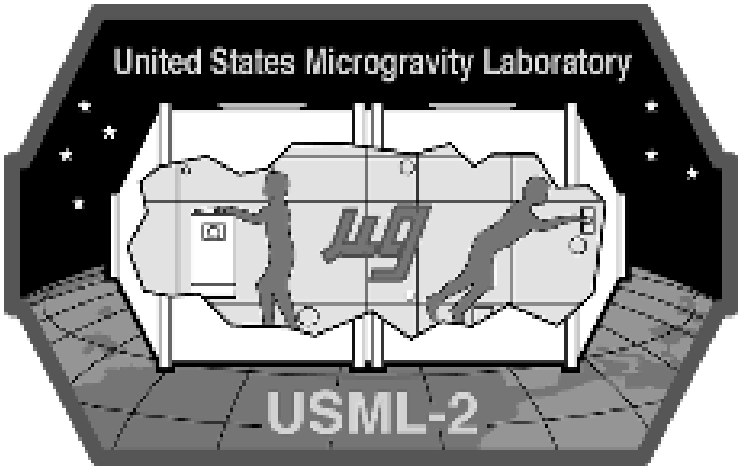
The Second United States Microgravity Laboratory

90-Day Science Report

THE SECOND UNITED STATES MICROGRAVITY LABORATORY

90-DAY SCIENCE REPORT

March 1996



This report summarizes the results from, as well as the status of, the investigations performed on the Second United States Microgravity Laboratory as of 90 days after landing. It was produced from inputs provided by the investigators and hardware developers and from mission records. It was reviewed for accuracy by the undersigned, the Assistant Mission Scientist, and the Program Scientist. This report should be considered as representing preliminary results from ongoing post-mission analyses and assessments and is an accurate summary account of the science activities carried out during the mission. A more comprehensive report is planned at a later date and will include more detailed results, representations, and illustrations and other visual data.

Investigators with the three acceleration measuring systems on this mission, the Orbital Acceleration Research Experiment (OARE), the Space Acceleration Measurement System (SAMS), and the Three-Dimensional Microgravity Accelerometer (3DMA), as well as the Microgravity Acceleration Work Station (MAWS), which provided the modeled acceleration environment, have been reducing their raw data and will be providing these data to the Principal Investigators upon request.

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Dr. Marcus Vlasse
USML-2 Mission Scientist

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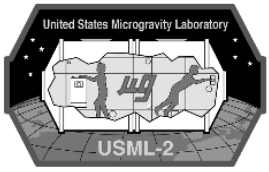


OVERVIEW

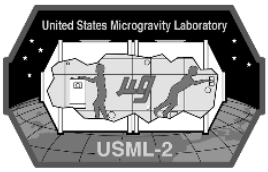
On October 20, 1995, the launch of the Space Shuttle *Columbia* with the Second United States Microgravity Laboratory (USML-2) mission continued the legacy of one of NASA's most successful scientific mission series. Using the knowledge gained from the USML-1 mission, scientists were able to prepare and improve their investigations and experiments by enhancing procedures, refining operations, modifying experiment hardware, and expanding methods for gathering data.

The 7-member crew performed USML-2 experiments around the clock to maximize the science on orbit. Scientists in the Shuttle and on the ground worked closely together before and during the mission to ensure proper understanding of each investigation's operations and to determine the best way to obtain the most data. It was a perfect example of interactive science in a unique laboratory environment.

The investigations that constituted the payload of the USML-2 mission covered these basic areas of microgravity research: Fluid Physics, Combustion Science, Materials Science, Biotechnology, and Technology Demonstrations. **Fluid Physics** research explores the behavior of fluids and the responses of fluids to the application or removal of different forces. **Combustion Science** research seeks to improve understanding of the basic combustion process and to determine how that process is affected by gravity, comparing results obtained in space with those obtained on Earth. **Materials Science** research increases the understanding of relationships between the structure, properties, and processing of materials. **Biotechnology** researchers attempt to grow protein crystals of sufficient size and perfection to determine their structure and formation, and to investigate the benefits of microgravity for growing cells and tissues. **Technology Demonstrations** help develop the hardware necessary for future microgravity research and refine existing hardware, making it more useful in ongoing experimentation.

Along with investigations that previously flew on USML-1, several new experiment facilities flew on USML-2. The **Advanced Protein Crystallization Facility (APCF)** is the first facility to use three methods of protein crystal growth: liquid-liquid diffusion, dialysis, and vapor diffusion. The **High-Packed Digital Television (HI-PAC)** Technical Demonstration gave scientists on Earth the ability to view multiple channels of real-time video and to monitor and change experiment parameters as needed to improve the quality and quantity of downlinked data, enhancing science returns. It also enabled the Principal Investigators (PIs) to take large quantities of video data of their experiments back to their research centers immediately after the flight. **Ground-to-Air Television** was first used on the USML-2 mission, allowing the scientists on the ground and the crew not only to talk to each other but also to see each other as they conversed about science operations. The **Geophysical Fluid Flow Cell (GFFC)** experiment, which studies how fluids move in microgravity, first flew on Spacelab 3 in 1985 and was extensively refurbished for this mission.

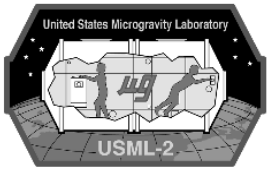
OVERVIEW



The experiments that measured the microgravity environment added to the success of the mission by providing a complete picture of the Shuttle's environment and its disturbances. The **Orbital Acceleration Research Experiment (OARE)** provided real-time acceleration data to the science teams. The **Microgravity Acceleration Workstation (MAWS)** operated closely with the OARE instrument, comparing the environment models produced by MAWS with the actual data gathered by the OARE. Two other instruments, the **Space Acceleration Measurement System (SAMS)** and the **Three Dimensional Microgravity Accelerometer (3DMA)**, took measurements throughout the mission, and those data will be provided to the science community when they have been analyzed.

The investigations performed on USML-2 brought together a large number of researchers from goverment, academia, and private industry, and combining the strengths of these communties allowed for more extensive ground-based research, advanced research techniques, improved microgravity experimentation, and a wider distribution of the knowledge gained in the process. The mission demonstrated that interactive science experimentation between the scientists on the ground and others in an orbiting laboratory not only is possible but also can be extremely successful in providing results of certain significance.

The preliminary results of the USML-2 experiments indicate that they were very successful. Summaries of each of the investigations follows.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY

Conditions on Earth limit the size and quality of protein crystals, but the microgravity environment of space was expected to allow the production of larger, more highly ordered crystals. The Advanced Protein Crystallization Facility (APCF) flown on USML-2 was the first facility designed to use three methods of protein crystal growth. The first method, liquid-liquid diffusion, or free interface diffusion, contained a protein solution and a salt solution that were separated by a buffer but were allowed to mix together slowly once the Shuttle was in orbit. The second method, dialysis, had the protein and salt solutions separated by a membrane. Vapor diffusion, or the hanging drop method, allowed crystals to form inside a drop of protein solution as solvent from the drop diffused to a reservoir. For all three methods, crystallization occurred at a constant temperature of 20 ˚C.

Video images were made of the crystals as they formed. The images should allow investigators to study the history of crystal development in microgravity. Investigators were particularly interested in why and how crystals nucleate to begin crystal formation. Investigators will analyze the crystals using X-ray diffraction, sophisticated detectors, and data processing equipment to determine the internal arrangement of their molecules.

Fifteen separate investigators used the Advanced Protein Crystallization Facility to study a wide variety of proteins.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of Apocrustacyanin C

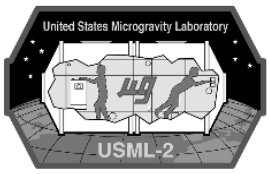
PI: N. Chayen

AFFILIATION: Imperial College, London, England

PURPOSE: The protein Apocrustacyanin C is a member of the lipocalin family of proteins, which binds to certain pigments that are widely distributed in plants and animals. Knowledge of the structure of the lipocalins will enable scientists to engineer these proteins to produce carriers that will bind more strongly to the pigment crocetin, which has anti-cancer properties.

RESULTS: There is a definite difference between the ground and flight crystals both in resolution enhancement and in the internal structures evaluated from rocking curve widths, which are a measure of the mosaicity of the crystal.

PRELIMINARY CONCLUSIONS: There is an indication of improvement in the space-grown crystals. The amount of improvement is now being analyzed and will be compared with results obtained with lysozyme experiments performed on the Second International Microgravity Laboratory mission.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystal Structure Analysis of the Bacteriophage Lambda Lysozyme

PIs: J.P. Declercq and C. Evrard

AFFILIATION: University of Louvain, Belgium

PURPOSE: The bacteriophage lambda lysozyme is a small protein of 158 amino acids. Like other known lysozymes, it is involved in the dissolution of the cell walls of bacteria. This enzyme is remarkable in that its mechanism of action is different from the classical lysozyme's mechanism; moreover, from the point of view of protein evolution, it shows features of lysozymes from different classes. After many years of efforts toward crystallization of the native enzyme, no suitable crystals could be obtained. Before this mission, different mutants also were tested, and it appears that the best results were obtained after replacement of the tryptophane residues by aza-tryptophanes, using the hanging drop technique. Investigators were able to grow only very small crystals of this mutant, even after seeding experiments. These crystals were too small for complete structure analysis but allowed determination of preliminary crystallographic data. The aim of this investigation was to produce well-ordered crystals suitable for high-resolution X-ray structure determination and analysis.

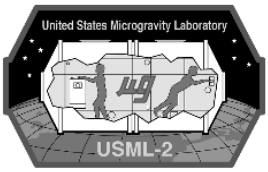
METHOD: The crystallization experiment of the bacteriophage lambda lysozyme was achieved using the vapor diffusion method. Five 8-ml handgun drop reactors were available for the experiment. Crystallization occurred at 20 ˚C in the same conditions used for the laboratory-grown crystals.

RESULTS: Crystallization of the protein occurred in two of the allocated reactors. Several very thin needles (Figure 1) were picked out but appeared to be smaller than the crystals grown on the ground and were, therefore, unusable for high-resolution X-ray structure determination and analysis. Precipitates were found in the other reactors.

PRELIMINARY CONCLUSIONS: It was concluded that the crystallization conditions of the bacteriophage lambda lysozyme seem to have changed during the microgravity experiment on USML-2. It can be assumed that it will be possible to optimize the microgravity crystallization parameters to obtain crystals of suitable size for X-ray analysis.

Figure 1. Very thin, needle-like crystals of bacteriophage lambda lysozyme grew in two reactors on USML-2.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS



Crystallization of the Visual Pigment Rhodopsin

PI: **W. de Grip**

AFFILIATION: University of Nijmegen, The Netherlands

PURPOSE: Visual pigments like rhodopsin are the primary photoreceptor proteins for a variety of light-regulated processes, such as vision, circadian entrainment, and photoperiodic reproductivity. Analysis of the protein crystals is needed to unravel the molecular mechanisms responsible for these processes.

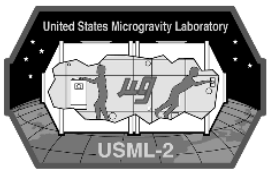
METHOD: Six hanging drop reactors were used on the USML-2 flight. With the hanging drop method, crystals form inside a drop of protein solution as solvent from the drop diffuses into a reservoir. All crystallization experiments occurred at 20 °C.

RESULTS: Some hardware anomalies occurred, and two of the six reactors partially or completely lost their hanging drops. Two other reactors did not achieve proper equilibrium, with almost no reduction in the volume of protein solution occurring.

Of the remaining two reactors, one had some small crystalline objects, and the other showed an abundance of small needle-shaped crystals, including some clusters. All objects were too small (<80 μm in the largest dimension) to undertake serious diffraction analysis.

Electroimmunoblotting analysis revealed that the needle-shaped objects contain rhodopsin, but this could not be unequivocally demonstrated for the other crystal shapes, possibly because the amount of protein was well below the detection level.

PRELIMINARY CONCLUSIONS: These results are less positive than those obtained with earlier flights. So far, the hardware problems are unexplained but do not appear to depend on the type of detergent used. Although crystal sizes are usually smaller in the ground controls, laboratory-grown crystals tend to grow larger upon prolonged incubation (4-12 weeks). The results recommend performance of crystallization experiments with rhodopsin under microgravity for more prolonged time periods (1-3 months).



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of the Protein Grb2 and Triclinic Lysozyme

PI: A. Ducruix

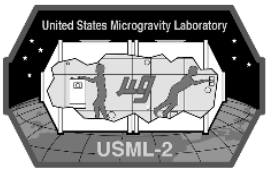
AFFILIATION: Laboratoire de Biologie Structurale, CNRS, Paris, France

PURPOSE: The initial intent was to use APCF reactors to study the influence of microgravity for two proteins that have been studied by the investigator group. The first of these is Grb2, an "adaptor" protein that binds to phosphotyrosine on several receptors on one side and on proline-rich domain of Sos, a guanine nucleotide exchange factor of Ras. Ground-grown crystals have given diffraction data only to 3.1 Å, and investigators expected better resolution from space-grown crystals. Six reactors were to be devoted to the Grb2 experiment. Unfortunately, the Grb2 samples proved to be unstable a few weeks before launch, leaving no time for another purification process. The reactors were returned to the European Space Agency.

Scientists also hoped to produce large crystals of triclinic hen egg white lysozyme for mosaicity, rocking curve, and topography studies. In the crystal growth field, lysozyme, an enzyme of 129 amino acids, has been one of the most studied proteins. An attempt was made to grow a tetragonal form of the enzyme aboard the Spacehab-1 mission. Although results were better than any previous space-grown attempts, there was no significant difference between those space samples (crystals diffracted to 1.3 Å) and ground-grown crystals. Using the quasi-planar wave produced by a four-reflections monolithic SI 220 crystal on the stationary wave station D25B at LURE-DCI, the investigator team recently performed direct measurements of rocking curves of reflections from tetragonal ground-grown crystals of lysozyme. The residual divergence of the monochromatic beam was close to 0.5 arc sec at lambda = 1.23 Å, and the relative bandpass was delta·lambda/lambda = 7.4×10^{-7}. Several crystals were essentially perfect single domains with rocking widths as small as 12 sec. The aim of the USML-2 experiment was to extend the study to the triclinic form of lysozyme, which presents the clear advantage of a smaller unit cell. Crystals "made in space" would be analyzed following the same procedure. At the same time, monoclinic crystals, which have a larger unit cell, would also be grown. Five reactors were devoted to this experiment, two for the triclinic crystals and three for the monoclinic ones.

METHOD: Hen egg white lysozyme is commercially available. Purity of the protein has been assessed by ion-spray spectroscopy. The isoelectric point is 11.3, and its molecular weight is 14305 $\pm$ 2 daltons for 129 amino acids. The protein is not glycosylated nor is there free cysteine, but there are three disulfide bridges. The unit cells (P1) of the samples to be flown were as follows: a = 34.2 Å, b = 31.8 Å, c = 27.1 Å, alpha = 111.6˚, beta = 108.3˚, and gamma = 88.8˚ with one molecule in the asymmetric unit and a = 27.96 Å, b = 60.61 Å, c = 62.94 Å, beta = 90.82˚ with two molecules in the asymmetric unit for the monoclinic form. The dialysis technique was used for both cases.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

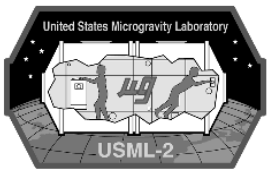


Crystallization of the Protein Grb2 and Triclinic Lysozyme (continued)

The volume of the protein solution was 450 microliters for all reactors. Transportation of the reactors was at 4 ˚C, but the flight experiments were performed at 20 ˚C. Ground controls were run in parallel in the laboratory using space reactors. The reactors were filled in Strasbourg, France, on September 19 and were activated on October 20. The protein is extremely stable, which proved invaluable to the experiment because of the numerous launch delays. The space-grown samples were sent to Strasbourg 2 days after landing.

RESULTS: Analysis in the laboratory showed that the reactors did not leak, as the salt concentrations were nominal. In addition, temperature data for the reactors were nominal. All five reactors maintained near nominal pH levels, and crystals grew in all reactors (both flight and ground controls). The APCF reactor operations, including activation, deactivation, equilibration of protein/reservoir solution, and thermal regulation, proceeded successfully. Recorded video images have not been received yet for analysis. Crystals of both monoclinic and triclinic lysozyme were immediately mounted in glass capillaries and were to have been subjected to X-ray radiation for diffraction limits and mosaicity measurements. Unfortunately, a long strike in France resulted in the shut-down of the French Synchrotron Radiation Facility, and the team's allocated beam time was canceled.

PRELIMINARY CONCLUSIONS: Analysis of the crystals will be performed in February when the facility reopens.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of RNA Molecules Under Microgravity Conditions

PI: V. Erdmann

AFFILIATION: University of Berlin, Germany

PURPOSE: Ribonucleic acids (RNAs) are essential macromolecules in living cells because they can assume not only structural functions (in the ribosomes) but also enzymatic functions (ribozymes) and can act as carriers of genetic information. This diversity has led to a new research field, called RNA-technologies, in which the structural and functional potentials of RNA are used in the areas of molecular biology, biotechnology, and medical diagnostics and therapy. To develop the RNA-technologies, it is essential that the structures of RNA molecules be determined at atomic resolution. The large size of RNA molecules limits structural determination to X-ray crystallography. One problem with the X-ray method is that it requires crystals of suitable size and quality for successful analysis. It is known that more than 20 different parameters influence the crystallization of biological molecules. Gravity is one of these influencing factors.

The goal of this APCF project was to analyze the influence of gravity on the crystallization of RNA molecules. The RNA molecules chosen for this study are ribosomal 5S RNAs (5S rRNAs) from *Thermus flavus*. The 5S rRNA molecules are essential components of the ribosomes, which are large RNA-protein complexes responsible for the synthesis of all cellular proteins. Since the 5S rRNA crystals obtained so far have exhibited a resolution of only 8 Å, which is not enough for X-ray analysis, the USML-2 experiment was designed to improve the quality of these crystals and to determine whether this improvement can be achieved under microgravity conditions.

METHOD: The crystallization experiments performed during the USML-2 mission consisted of *Thermus flavus* 5S rRNA species, which had been engineered at their 3’- and 5’-ends in such a way that their structures were more stable than the wild-type molecule. It was anticipated that the structurally stabilized 5S rRNA variants would be better suited for the crystallization and X-ray analysis.

The crystallization experiments were performed in 5 APCF reactor chambers (15 µl volume). The crystallization method used was microdialysis.

RESULTS: Of the five crystallization experiments performed, three yielded crystals. The crystals obtained were larger in size and more numerous than those obtained in simultaneous ground-control experiments. The largest space-grown crystals exhibited a length of 0.7 mm. In the ground-control experiments, only two chambers yielded crystals. These were smaller in size and less numerous than those grown in space.

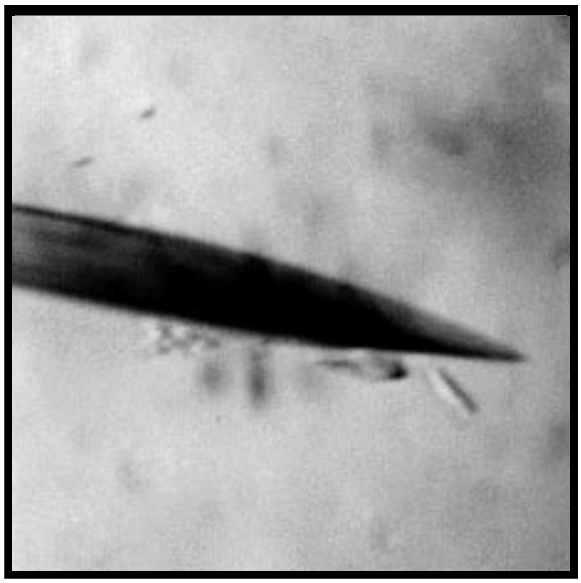
ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of RNA Molecules Under Microgravity Conditions (continued)

The largest crystal had a length of 0.45 mm. Figure 1 shows samples of the ground- and space-grown crystals.

All crystals were analyzed by synchrotron radiation at the DESY facility in Hamburg, Germany, 6 days after landing. Both space and ground-control crystals exhibited a resolution of 13 Å.

PRELIMINARY CONCLUSIONS: The results show that the crystallizations performed in space yielded more and larger crystals than those in the ground experiments. The fact that the space crystals did not exhibit a better resolution by X-ray analysis may be connected to the last launch delay. During the 7-day delay, the RNA samples were stored at 20 ˚C. Ground-control studies have shown that an 11-day storage period at 20 ˚C caused a yield in only 2 of 5 crystal growth chambers. Storage at that temperature for 4 days has shown crystal growth in 4 of 5 chambers. The results, therefore, imply that an extended storage of the RNA molecules at 20 ˚C will have a negative effect upon the crystallization process. Better results are anticipated in future missions in which the nominal period of storage (4 days) is not exceeded.



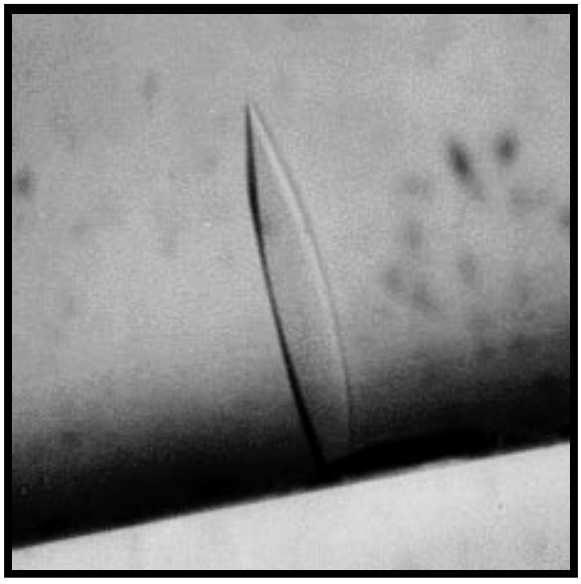
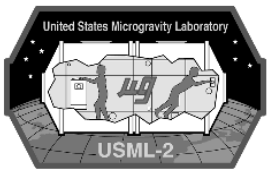


Figure 1. Crystals obtained from *Thermus flavus* engineered 5S rRNA molecules grown under microgravity conditions (top) and in a laboratory control experiment (bottom).



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Microgravity Crystallization of Thermophilic Aspartyl-tRNA Synthetase and Thaumatin

PI: R. Giegé

AFFILIATION: CNRS, Strasbourg, France

PURPOSE: Crystallization of the plant sweetening protein, thaumatin, and the thermophilic aspartyl-tRNA synthetase (AspRS) from *Thermus thermophilus* has been studied under microgravity conditions. Thaumatin is a plant protein that produces an extremely sweet tasting sensation in microgram quantities when it is orally consumed by humans. Thaumatin is nontoxic, non-carcinogenic, and naturally low in calories, which makes it a potentially strong substitute for common table sugar. The aspartyl-tRNA synthetase is an enzyme that mediates the action of translating genetic information. In the interest of exploring further structural information that could be extended to other synthetases, scientists have searched for novel crystallization conditions that would give rise to crystals of higher resolution or of alternative crystal forms with enzyme structural variance. The crystallization conditions of both proteins are known and their structures have been solved. Thaumatin and AspRS are well suited to serve as model proteins for crystallization studies. The structures of crystals grown in space can be readily compared to the corresponding results obtained on Earth.

Previous experiments have demonstrated that, in at least in some cases, macromolecular crystals of improved quality can be grown in a microgravity environment. The fundamental objective of this investigation is to obtain protein crystals in microgravity, to determine if the influence of microgravity affects crystal growth, and to observe any anomalies or enhancement in growth that may implicate a gravity effect.

METHOD:

I. Thaumatin: Thaumatin was purchased from Sigma Biochemicals and used directly without any further purification. Five APCF dialysis (DIA) reactors were used to investigate crystal growth under microgravity conditions. These include four 188-ml DIA reactors, each one containing thaumatin solutions in the protein, buffer, piston T-type, and salt chambers. A 67-ml DIA reactor with the same chamber components also was used, but it included different protein concentrations.

Ground controls for the 188-ml DIA reactors were prepared in exactly the same manner as the microgravity experiments and underwent the exact same transport and pre-launch conditions, including the launch delay time. The 67-ml DIA ground-control reactor was prepared under the same conditions and was activated for the same period of time as the one that was in space, except that it was not executed in parallel with the time of the flight as the others because of the lack of reactor availability.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Microgravity Crystallization of Thermophilic Aspartyl-tRNA Synthetase and Thaumatin (continued)

II. Aspartyl-tRNA synthetase: Thermophilic AspRS has been cloned and overexpressed in *E. coli.* The protein was purified in a three-step procedure, including a flocculation at 70 °C and two chromatographies on DEAE-cellulose and hydroxyapatite columns. Starting with 50 g of overexpressing *E. coli* cells yields about 50 mg of pure enzyme. As estimated by activity assays, gel electrophoresis, and dynamic light scattering, the enzyme is pure and conformationally homogeneous.

AspRS was prepared in three 67-ml dialysis and two 80-ml hanging droplet reactors. The dialysis reactors were prepared as described above. The protein chamber contained 10 mg/ml in 5% saturated sodium formate in 25mM Tris-HCI pH 7.5, 1 mM $MgCl_2$, and 0.1 mM EDTA. The buffer volume contained the same reagent as the protein chamber without the enzyme. The piston and salt chambers contained 30%, 35%, and 40% saturated sodium formate in 25 mM Tris-HCI pH 7.5, 1 mM $MgCl_2$, and 0.1 mM EDTA in each respective reactor. In the hanging droplet reactors, 10 mg/ml of AspRS in 12.5% and 15% saturated sodium formate mixed with 25 mM Tris-HCI pH 7.5, 1 mM $MgCl_2$, and 0.1 mM EDTA were set to equilibrate to 25% and 30% saturated sodium formate respectively in the two separate hanging drop reactors.

RESULTS:

I. Thaumatin

A. Size and number of crystals: The average size of protein crystals grown in space was significantly larger than that of the corresponding Earth-grown control crystals. It is worth noting that the crystal size and its consequential analysis discussed in this summary are compared only to the corresponding Earth controls and not to the best crystals ever grown on the ground. Generally, the crystals were about five times larger than their corresponding controls. The largest crystals measured 1.7 mm in their longest dimensions. This was about twice as big as crystals grown on Earth under the same conditions. Many of the crystals grown in these reactors were found to be attached to the dialysis membrane or to the chamber walls.

B. X-ray analysis: Crystals grown under the reported conditions on Earth and in space are tetragonal and belong to the space group $P4_12_12$. Data were collected with a Siemens area detector and were evaluated on line with the program XDS or at the European Synchrotron Radiation Facility, Grenoble, France. Processing and data analysis were performed with the software DENZO and the CCP4 package suite.

Microgravity Crystallization of Thermophilic Aspartyl-tRNA Synthetase and Thaumatin (continued)

1. Mosaicity: Crystal mosaicity was evaluated by measuring the size and shape of the Laue diffraction spots. Four crystals were evaluated, two microgravity-grown and two Earth-grown. Medium intensity diffraction spots were collected in all cases to avoid any flaring of spots. Representative samples of reflections at various Bragg angles were scanned, and the full widths at half maximum intensity were quantified. It appears that crystals grown in space have lower mosaicity than those grown in laboratories on the ground. These measurements were made on a synchrotron beam line assumed to have an almost parallel beam, such that the angular width of the diffraction profile would be dominated by the mosaicity of the crystal rather than the X-ray beam divergence or spectral spread. A two-dimensional profile of these results is underway.

2. Resolution limits: The resolution limits of both space and ground crystals were about the same, diffracting as far as 1.6 Å resolution. The differences between space and Earth crystals were marginal.

II. Aspartyl-tRNA synthetase

None of the reactors that contained AspRS contained any crystals of diffractable size. Most of the reactors contained slight precipitation, and in one of the dialysis reactors, very small crystals were observed. These small crystals had an estimated size of less than 0.050 mm. We have speculated that the activity of this particular enzyme did not withstand the flight delay of this mission.

Even though AspRS is known to retain its activity longer than most enzymes, the observation of precipitation suggests that the protein had denatured during the extended delay. This was also observed in the ground-control reactors where the pre-activation and activation conditions were treated as identically as possible. Earlier ground-control tests did produce crystals under the same crystallization conditions, where the duration of pre-activation step was not as long.

PRELIMINARY CONCLUSIONS: The quality of the space crystals obtained in this study is compared directly to those grown under ground conditions at the same time. Space- and Earth-grown crystals were prepared from the same protein preparation and have undergone the same manipulation, so that the relative comparisons between crystals grown in space and on Earth can be significant.

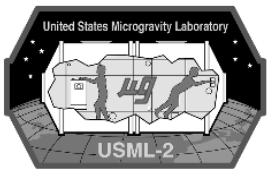
Thaumatin crystals grown in the DIA reactors in space were observed to grow much bigger with significantly less nucleation than the corresponding ground controls. This observation is most striking and is statistically significant, since this phenomena has been observed in all the reactors containing thaumatin. The diffraction limits of the crystals grown in space were about the same as those grown on Earth; however, the mosaicity of space-grown crystals was less than that of the ground controls.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Microgravity Crystallization of Thermophilic Aspartyl-tRNA Synthetase and Thaumatin (continued)

Very small crystals were obtained for AspRS, but these crystals were not big enough for analysis. Most reactors containing this enzyme did not crystallize. Investigators speculate that the flight delay may have attributed to the denaturation of the protein, thus hindering its normal crystal growth.

Overall, results indicate a significant difference between quality and size of thaumatin crystals that are grown on Earth and those that are grown in space.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization in Space of Designed and Natural (alpha/beta)-Barrel Structures

PI: **J. Martial**

AFFILIATION: University of Liege, Belgium

PURPOSE: This experiment pursues crystallization assays with artificial and natural (alpha/beta)-barrel proteins.

I. New versions of octarellins (*de novo*-designed alpha/beta-barrel proteins): The resolution of *de novo* structures using crystallization and X-ray diffraction provides the most critical test for the concepts and principles used to guide protein design. This experiment should provide information and enhance current knowledge of protein folding processes. This project should enable the integration of tri-dimensional structural data into the building of the third generation of octarellins. This goal is unrealistic without a correct tri-dimensional structure; determining this structure requires high-quality crystals.

II. Triosephosphate isomerase (TIM): This glycolytical enzyme is known to exist in nature as a dimer, or two identical alpha/beta-barrel subunits. First, crystallization assays are performed on human TIM (hTIM) mutated in the laboratory to form a stable monomeric alpha/beta-barrel protein. Second, crystallization assays are performed with TIM isolated from the hypertherophilic bacterium, *Thermotoga maritima*.

The analysis of these alpha/beta tri-dimensional structures (a monomeric and thermostable TIM barrel) is crucial to determining the structural parameters leading to stable alpha/beta-barrel folds. Knowledge of these parameters will contribute to the design of a more stable octarellin fold. The microgravity environment should enable investigators to grow the high-quality crystals needed.

METHOD: The production and thorough purification of at least 100 mg of various proteins to be studied (octarellins, the hTIM native and mutant enzyme, and the *Thermotoga* TIM) were done in the laboratory. Preliminary crystallization assays were performed in the laboratory, and, based on this screening, various conditions were selected for the different Hanging Drop (HD) (80 µl) reactors and Fluid Interface Diffusion (FID) (200 µl) reactors.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization in Space of Designed and Natural (alpha/beta)-Barrel Structures (continued)

RESULTS:

I. Octarellin Crystallization:

Reactor FID 312: Bottom solution 8% PEG 6000; 100 mM Tris-HCl (pH 8.5)/octarellin II: 10.1OD

Needle-shaped microcrystals were obtained in both the ground-control and flight-unit samples. Three small crystals, approximately 10 microns in diameter, grew on Earth and diffracted to below 3 Å. These crystals were quite resistant to radiation damage. With these three crystals, 70 degrees of data were collected. One crystal was obtained during the flight.

Reactor HD 107: Bottom solution 25% PEG 5000; 0.01M $NiZnSO_4$; 100 mM MES (pH 6.5)/ octarellin III: 2.7OD

Octa II did not give any crystals on Earth or in space.

II. *Thermatoga* TIM Crystallization

Reactor HD 184: Bottom solution: 2% PEG 400; 2M $(NH_4)_2\ SO_4$; 100 mM HEPES (pH 7.5)/ Thermo TIM: 1.6mg/ml

Many small and very regular crystals were obtained, and this crystallization was recorded on videotape. These crystals have been used for diffraction assays. The Earth-grown crystals diffracted to a resolution below 2.3 Å, in contrast to 3 Å for the space-grown crystals. The crystals were very sensitive to radiation damage. After one 5-second exposure, the diffraction decreased to 3 Å and below. As a result, only a partial data set could be collected.

Crystals also were obtained in the ground-based reactor, with two types being present: regular crystals with the same form as the space-grown crystals and needle crystals. These crystals also have been used for diffraction.

III. Human TIM crystallization (native enzyme and mutated enzyme)

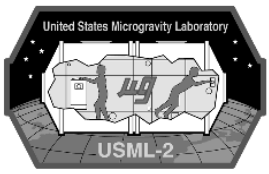
Reactor FID 305: Bottom solution 15% PEG 5000; 100 mM MES (pH 6.5)/human TIM (wild type): 14.02 OD

No regular crystals were obtained for the native human TIM.

Reactor FID 311: Bottom solution 15% PEG 5000; 100 mM MES (pH 6.5)/Monomeric mutated enzyme: 2.26 OD

No regular crystals were obtained with this enzyme.

PRELIMINARY CONCLUSIONS: Crystals of *Thermotoga* TIM suitable for X-ray data collection were obtained in space, and a clear difference in the form of these crystals was evident when compared to the Earth-grown crystals. The effect of microgravity on the crystal morphology has yet to be investigated.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of Turnip Yellow Mosaic Virus, Tomato Aspermy Virus, Satellite Panicum Mosaic Virus, Canavalin, Beef Liver Catalase, Concanavalin B, Thaumatin

PI: A. McPherson

AFFILIATION: University of California, Riverside, California

PURPOSE: Several crystals were grown and studied to determine the effects of microgravity on protein crystal growth by evaluation of the size, habit, quality, defects, and diffraction properties.

METHOD: This investigation involved performance of the liquid-liquid diffusion growth method in 12 crystallization reactors. The reactors were activated shortly after orbit was achieved and were deactivated just before re-entry. Three of the cells were monitored periodically, using the APCF video system. (These recordings are not yet available for study.) The 12 cells were transported back to the University of California at Riverside (UCR) after photography at Kennedy Space Center in a thermally controlled container. They were re-photographed, opened and unloaded, and then photographed again. Some X-ray diffraction data were collected on the thaumatin crystals.

RESULTS: Overall, the USML-2 mission was disappointing for this investigation, with few crystals grown at all. Only thaumatin grew high-quality crystals with sizes as large or larger than those grown on Earth. The quality of the thaumatin crystals was excellent in all cases, except those where the crystals grew with faces against walls. These showed extensive striations. One thaumatin crystal grew from the wall from the point of its tetragonal face and was safely returned to UCR with no evidence of perturbation or damage. The thaumatin crystals increased in size with increasing protein concentrations and diffracted strongly to the maximum resolution that our data collection system could achieve, approximately1.5 Å. The team is anxiously awaiting the arrival of the videotapes from the European Space Agency, as one of the best thaumatin samples was under video observation.

For the colored proteins (ferritin, catalase), the mid-channel was hardly colored, indicating that back diffusion of the protein was virtually zero. There were numerous bubbles in the samples, in spite of days of topping off the samples.

PRELIMINARY CONCLUSIONS: The APCF system worked well for the thaumatin crystals, so it cannot be faulted on fundamental design. The launch delay was probably detrimental to the validity of the samples, and the long waiting period before launch probably degraded the experiment. For future investigations, very high protein concentrations and lower precipitant concentrations should be used, as these conditions produced the best quality crystals. Crystals grown in the APCF cells were not damaged physically or perturbed by re-entry and unloading.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of Photosystem I

PIs: W. Saenger and P. Fromme

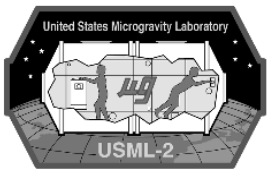
AFFILIATION: Technische Universität and Freie Universität, Berlin, Germany

PURPOSE: In all green plants and cyanobacteria, two protein complexes are involved in photosynthesis: photosystem I and II (PSI and PSII). PSI, from the thermophilic cyanobacterium *Synechococcus elongatus*, consists of 11 polypeptide chains, about 90 chlorophyll *a* molecules, and three Fe_4S_4 clusters and occurs *in vivo* as a trimer. PSI has been crystallized, and the X-ray structure analysis has provided an electron density map at 4 Å resolution. The crystals show a large mosaic spread of >1˚. It was hypothesized that this large spread could be reduced by growing the crystals in microgravity. The USML-2 PSI crystal growth experiments were conducted in an attempt to reduce the mosaic spread, thereby increasing the resolution of the diffraction data.

METHOD: PSI was dissolved at a concentration of 80 mg/ml in 100 mM $MgSO_4$, 5 mM MES-buffer pH 6.4, 0.02% beta-dodecylmaltoside. In the APCF, this solution was dialysed against a buffer with reduced salt concentration (with an end concentration of 11 mM $MgSO_4$) at 20 ˚C. Because the flight was postponed, the dialysis cells had to be filled twice to have the material as fresh as possible for the crystallization experiment.

RESULTS: The results of the experiment are very encouraging. On Earth, the largest of the hexagonal rod-like crystals grew on the dialysis membrane and was 2 mm long and 0.5 mm Ø (volume of 0.4 mm^3). In space, the crystals grow to 4 mm long and 1.5 mm Ø (volume of 7 mm^3). A temperature of 20 ˚C was required for technical reasons, but the optimum temperature for growth of PSI is 4 ˚C. The diffraction quality of the crystals decays with time and is worse at 20 ˚C than at 4 ˚C. In spite of this, the crystals still diffracted to 3.8 Å, and the mosaic spread reduced slightly to approximately 0.7˚. A data set was collected and is currently being evaluated.

PRELIMINARY CONCLUSIONS: The experiment was very successful, but it should be repeated at the optimum temperature of 4 ˚C. This should produce crystals that would have a lower mosaic spread and should permit collection of X-ray diffraction data at higher resolution than with Earth-grown and previous space-grown crystals.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of Glutathione S-Transferase in Microgravity

PI: L. Sjölin

AFFILIATION: University of Göteborg, Sweden

PURPOSE: The primary goal of the 4-4 GST studies includes increasing the size, improving the resolution, and reducing the nucleation of the crystals. Investigations involve a protocol similar to that carried out for the previous RNase S crystallization studies that recently have been completed. It is believed that improvement of these characteristics would allow the structure determination of this important mu isozyme. Design of suitable experiments to optimize each of these parameters is necessary, along with appropriate control experiments.

METHOD: The hanging drop method of crystallization was used to grow glutathione S-transferases (GST; EC 2.5.1.18), a family of enzymes involved in the detoxification of endogenous and xenobiotic electrophilic substances. These proteins catalyze the nucleophilic addition of glutathione (GSH) to substrates bearing electrophilic functional groups. The dimeric cytosolic enzymes, which are composed of subunits with a molecular weight of 25,000, have been classified into four distinctive isoenzymatic species designated alpha, mu, pi, and theta. Sequence identities between enzymes in different gene classes range between 25% to 35% and between isozymes in the same gene class from 60% to 80%. Heterodimers may form between different isozymes from the same gene class, but intergene class heterodimers have not been observed.

The glutathione-binding site (G site) of all known GSTs is primarily associated with the N-terminal domain at one end of the antiparallel beta-sheet with beta-strands found in a beta2, beta1, beta3, beta4, arrangement. Beta1 and beta2 are parallel, and beta3 is antiparallel to beta1 and beta4. The GSH binding site is near theC-terminal ends of beta1 and beta2. GSH binds in a similar manner in each of the structures that have been determined, but the specific details vary significantly, primarily because of the large variation in the sequence.

RESULTS: The reactors were inspected immediately after the flight, and small, irregular crystals were found in three of the four reactors. These crystals were of poorer quality than other crystals grown on Earth using regular laboratory equipment. X-ray analysis was not feasible on these crystals. Instead, some effort has been spent on understanding why the crystallization experiment in space evidently gave crystals of poorer quality than similar crystallization experiments in the laboratory.

It appears the reason that good, diffraction-quality crystals were not achieved is ultimately related to the reactor design. Also, the wicks were nearly dry when the reactors from the Shuttle were opened. This assessment is based on the following:

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of Glutathione S-Transferase in Microgravity (continued)

I. The same protein under the same sample conditions yielded untwinned, birefringent, diffraction-quality crystals in plates of sitting drops using regular laboratory equipment.

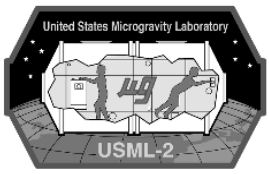
II. The control reactors had the same sample conditions except that the wicks were saturated with precipitant instead of using only 350 ml, and they yielded many twinned plates that grew on the wall of the reactor at the interface between the teflon plunger and the plastic wall of the cylinder. These crystals were thicker and less twinned than the microgravity crystals but still were not diffraction quality. Drying of the wicks in the microgravity samples cannot fully explain the poor quality of the crystals, though the drying seems to have caused more twinning and is somewhat detrimental.

III. The microgravity crystals grew from the interface between the plunger and the cylinder wall. The reactor design results in initiation of crystallization at this interface.

PRELIMINARY CONCLUSIONS: The solutions were evidently good, so if the problems result from the reactor design, the major differences between the sitting drop plates and the reactors are because of the container surfaces. The sitting drop bridges used in the regular laboratory experiments are very smooth and are highly polished. The reactor surfaces may be pitted along the top edge of the cylinder wall, because of its contacts when sealed, but are in contact with the drop when activated. The plastic of the reactor wall is a different material than the highly polished, sitting-drop bridge. The teflon surface also could be allowing multiple initiations of the crystals, though the crystals are located at the interface with the cylinder, implying that initiation cannot start on the teflon.

The crystallization solutions were very complex (seven components in the drop and three components in the reservoir/wicks), and this might have resulted in unpredicted interactions of the solutions with the reactor plastic/teflon or the wick material. A major difference between the crystallization plates and the reactors is that the coverslips are sealed with grease and the reactors have a rubber gasket. This may result in a difference in the quality/integrity of the seals, which could affect both the vapor diffusion and the rate at which the wicks dry out (though one would have expected one of the other groups to notice this also). Finally, the precipitant may not be vapor-diffusing as efficiently or at the same effective concentration as the wicks, compared to the well solutions of the plates.

The recommendations are to change the protocol to saturate the wicks and to modify the reactors with more highly polished, smooth reactor cylinder and plunger surfaces. It would also be good to study the quality of the seals, perhaps by putting a known amount of solution inside a sealed reactor to determine whether it dries out over time.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Protein Crystal Growth: Light-Driven Charge Translocation Through Bacteriorhodopsin

PI: **G. Wagner**

AFFILIATION: University of Giessen, Germany

PURPOSE: Small amphiphilic membrane proteins, such as bacteriorhodopsin (BR), that have small surface protrusions extending through the cell membrane will be embedded in micelles after detergent solubilization. Detergent-solubilized BR molecules tend to form filamentous crystals like micelles do in the hexagonal phase. The contacts that cause the BR filaments to pack together and form a multi-crystalline cluster are hydrophilic interactions between the loop regions of protruding BR molecules in aligned filaments. The hydrophilic interactions are weak and easily disturbed, resulting in considerable disorder in the BR crystalline array in the presence of convective turbulence and sedimentation. The goal of the experiment on USML-2 was to minimize these gravity-related effects.

METHOD: In a centralized European laboratory (Hamburg), identical test solutions were divided in half and filled into pairs of identical APCF liquid-liquid diffusion reactors to be transported to the USA for installation into the USML-2 Spacelab (microgravity experiment) or for use as ground controls. Habit and uniformity of the crystals grown were determined by measuring the length and diameter of each crystal along the square face of the protein chamber of the APCF reactors after the mission. Following crystal harvesting and mounting, either in X-ray capillaries or in cryofiber loops, resolution limits were determined from diffraction images of synchrotron radiation.

RESULTS: In a new experiment protocol, first used under microgravity conditions during the USML-2 mission, both the compact alignment of the crystalline filaments of BR and the increase in crystal size in microgravity, as reported earlier [Wagner, G. (1994) ESA Journal 18, 25-32], were greatly improved and resulted in a considerable increase in diffraction power. Close to the micellar consolution boundary, the molecular rods of BR were tightly packed together, and the crystal morphology exhibited smooth surfaces and sharp edges in cubic or needle-shaped habits of up to 1 mm in length.

PRELIMINARY CONCLUSIONS: The new, good-quality BR crystals, grown in microgravity and on the ground, combined with excellent synchrotron facilities, allow data collection of the BR crystals that were shown recently to diffract to a resolution limit of up to 3.8 Å.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization of the Epidermal Growth Factor (EGF) Receptor

PI: W. Weber

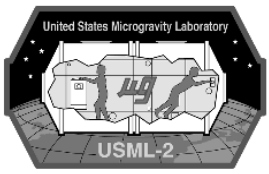
AFFILIATION: University of Hamburg, Germany

PURPOSE: The EGF receptor is the prototype of a family of tyrosine kinase receptors involved in cell growth control. Many human malignancies are characterized by its overexpression. The solution of the EGF receptor structure would pave the way for drug design and novel concepts of therapeutic treatment of tumors; however, the crystal structure of none of the growth factor receptors has been obtained thus far. The difficulty of crystallizing a membrane protein has been overcome by purifying only the hydrophilic external domain of the EGF receptor. Using this ectodomain, the co-crystallization with the ligand EGF was achieved; diffraction of these crystals had been poor, probably because of the high amount of heterogeneous carbohydrate (30% of molecular mass). Microgravity conditions have been found to favor crystal growth.

METHOD: On USML-2, various forms of EGF receptor protein were flown, differing in glycosylation and in pI values. Hanging drop reactors and dialysis reactors were used. Except for some setups with micro-crystals, only one hanging-drop reactor yielded crystals suitable for X-ray analysis. It contained about 10 crystals of 0.3 mm diameter. The EGF receptor protein used in this reactor had been biosynthetically modified in its glycosylation. Compared to the wild-type receptor, the carbohydrate of this form was rich in mannose rather than in complex structures. This modification may reduce microheterogeneity and is assumed to favor crystal growth.

RESULTS: Diffraction data from three crystals were collected using the synchrotron beam line BW6 at the DORIS storage ring, DESY Hamburg. The storage ring operated in the main user mode with 4.5 GeV and up to 100 mA. Images were recorded on the 300-mm MAR Image Plate scanner at 4 ˚C and at room temperature. Exposure times were in the range of 3 to 5 minutes for 1.5 degree rotation using a wavelength of 0.96 Å. The crystal-to-plate distance was set to 600 mm. The diffraction of all crystals analyzed was comparable: maximum resolution was 6 Å with a remarkably high quality of spots. The space group $P2_12_12$ could be evaluated using the DENZO processing package.

PRELIMINARY CONCLUSIONS: These results confirmed previous data obtained on STS-47. The best Earth-grown crystals had yielded comparable results but required larger sizes and much more time to grow. EGF receptor crystallization, therefore, seems to benefit from microgravity conditions. Further modifications of receptor glycosylation are now being generated and will be tested to improve crystal quality.



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Crystallization in a Microgravity Environment of CcdB, a Protein Involved in the Control of Cell Death

PI: L. Wyns

AFFILIATION: Free University of Brussels, Belgium

PURPOSE: CcdB is a protein involved in the control of cell death. The CcdB-mediated cell killing involves poisoning of DNA-topoisomerase II complexes. It converts the wild-type gyrase into a DNA-damaging agent. Poisons of eucaryotic topoisomerase are regarded as potent candidates for anti-cancer drugs. Elucidation of the structure and the mode of action of the CcdB protein may lead to the design of new antibiotics and anti-tumoral drugs. Efforts to crystallize CcdB on Earth led to the identification of experimental conditions for the growth of several crystal forms. The objectives of the microgravity experiments were the following:

I. Improvement of crystal quality and an attempt to solve a systematic twinning problem

II. Crystallization of a specific double mutant (Gly70Cys and Glu77Gln), which did not produce Earth-grown crystals large enough for data collection.

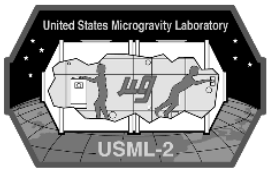
METHOD: The wild-type CcdB was used in one Hanging Drop (HD) reactor and one Fluid Interface Diffusion (FID) reactor. The mutant CcdB was used in one HD and two FIDs. The conditions used for these flight APCF reactors were based on knowledge gained from crystallization experiments performed on Earth.

RESULTS:

Wild-type CcdB: The ground-control reactor did not provide any crystals in either the HD or FID reactors. The team had been unable to obtain any CcdB crystals in an FID ground setup. The major problem was the appearance of precipitation immediately after activation of the reactor. In contrast, under the microgravity conditions of the USML-2 mission, CcdB crystals were obtained in both the HD and FID reactors. During investigation of the crystals after the flight, it was found that twinning was still present, although single crystals were obtained in the FID reactor.

Mutant CcdB: In the ground-control experiment, small needle-shaped crystals were obtained in the HD, as well as in the salt chamber of the FID reactor. In the space HD reactor, the same amount of crystals was obtained but they were smaller in size. No crystals were obtained in the space FID reactor; however, postflight activation of this reactor resulted in a crystallization (in the salt chamber) comparable to the ground control.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS



Crystallization of Ribosomes

PI: A. Yonath

AFFILIATION: Max-Planck Laboratory for Ribosomal Structure, Hamburg, Germany

PURPOSE: Ribosomes are the universal cell organelles facilitating the translation of the genetic code into polypeptide chains. The prokaryotic ribosomes are assemblies of a total molecular weight of 2.3 x 10^6 daltons, containing up to 73 different proteins and three RNA chains with about 4500 nucleotides, arranged in two subunits of unequal size. Aiming at the elucidation of the molecular structure of the ribosome, we have grown single crystals from functionally active ribosomes, as well as their complexes with components of protein biosynthesis and their natural, mutated, selectively depleted, and modified subunits. In contrast to the natural tendency of ribosomal particles to disintegrate, the crystalline ribosomal particles maintain their integrity and functional activity for long periods. X-ray crystallography data are collected from the ribosomal crystals at cryo-temperature (85 K), using intense synchrotron radiation sources. The best crystals diffract almost to atomic resolution (2.9 Å).

All ribosomal particles display a marked tendency to form very thin crystals, which tend to fracture, split, or crack upon handling, causing a loss of precious synchrotron radiation time and severe difficulties in data collection and evaluation. Because investigators believe that the formation of thin plates is influenced by the contact of the crystal's nuclei with flat surfaces, they assumed that the crystals would become thicker under microgravity conditions. Hence, the aim of the USML-2 experiments was to grow ribosomal crystals of favorable morphology and high quality.

The long-term goal is to elucidate the molecular structure of ribosomes by X-ray crystallography. Crystals of intact ribosomal particles suitable for such studies have been grown in the ground-based laboratory. Data collection became feasible by the introduction of cryo-temperature, since upon irradiation at ambient temperatures, the ribosomal crystals deteriorate instantly.

The investigator team has observed consistently that significantly better crystallographic data could be collected from thicker crystals, which are only occasionally grown. Much effort, therefore, has been invested in increasing the thickness of the crystals. However, experiments growing crystals in the laboratory revealed that this aim was not routinely accomplished. It is assumed that the tendency of the crystals to form thin plates is influenced by the contact of their nuclei with the float surfaces of the growth chambers. Under microgravity conditions, the crystals are supposed to float freely and their contact with flat surfaces reduced or even eliminated, facilitating the growth of thicker crystals. The intent of this experiment was to use microgravity not only for extending the morphology and the size of the crystals but also for further improvement of their internal order and mechanical properties. The goal was to obtain

Crystallization of Ribosomes (continued)

ribosomal crystals of a low mosaic spread, which would diffract well and to higher resolution limits than that of the current Earth-grown crystals. These crystals were expected to yield crystallographic data of high quality.

METHOD: In preparation for USML-2, several test experiments were run, screening for the particular conditions that are most suitable for each of the ribosomal preparations. To mimic growth under microgravity, the geometric parameters of the chambers used for crystal growth and the densities of the growth solutions were varied systematically.

In previous crystallization experiments performed on Earth, it was determined that the time needed to reach equilibrium within the crystallization chambers plays a crucial role in the formation of quality crystals from ribosomal particles; therefore, test experiments were performed on the ground in an attempt to optimize this parameter. The crystallization conditions were modified systematically to isolate and select the most promising ones.

The vapor diffusion method of growth was used, attempting slow equilibration of small droplets within their reservoirs. The samples were prepared immediately before delivery to Kennedy Space Center and were inspected right after they were returned. The crystallization mixtures and reservoirs were prepared with the same compositions that have proven to be suitable on Earth. The space-grown crystals will be irradiated by a bright synchrotron X-ray beam at cryo temperature, and the crystallographic data collected will be evaluated and analyzed by the standard procedures, as well as by those developed by the investigator team.

Also, an attempt was made to grow ribosomal crystals on Earth under conditions mimicking microgravity. Investigators constructed crystallization solutions with densities similar to that of the crystals and attempted crystallization in these solutions, assuming that the crystals, once formed, would float. In parallel, they attempted to grow crystals within gels. None of these attempts were successful, presumably because the compositions of the modified crystallization solutions were not suitable for crystal growth.

RESULTS: Almost every droplet yielded crystals even without seeding, which is a crucial requirement for the growth of quality crystals on Earth. Of special importance is the morphology of the crystals. Although still too small for X-ray crystallography, a few crystals grown in space are of somewhat better proportions than those grown on Earth and have a more isotropic shape, indicating the potential of microgravity. In addition, almost all crystals grown in space are rather round, a property never observed on Earth.

It is noteworthy that most of the ribosomal crystals did not break upon return to Earth. This is most important for this experiment, since the crystals are much more fragile and delicate than those of average size proteins.

ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Microgravity Crystallization of *Sulfolobus Solfataricus* Alcohol Dehydrogenase

PI: A. Zagari

AFFILIATION: Biocrystallographic Center, University of Naples, Italy

PURPOSE: Alcohol Dehydrogenase, ADH, is an enzyme that occurs in large amounts in the liver of mammals, where it plays an important role in various physiological functions, including the breakdown of ethanol. Mammalian ADH is unstable at high temperatures or in the presence of organic solvents. These properties severely limit its biotechnological applications in the synthesis of organic compounds. On the other hand, ADH from *Sulfolobus solfataricus* (SsADH), an archaeon that thrives at high temperatures, has greater thermal stability and greater resistance to organic solvents, detergents, and other denaturing agents. Given these peculiar and unusual properties, the enzyme is a good candidate for industrial applications.

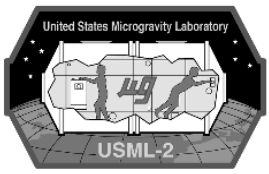
Greater knowledge of the three-dimensional structure of this enzyme may define the structural factors responsible for its stability. As a consequence, protein engineering and site-directed mutagenesis experiments could be planned to produce the enzyme with altered and optimal properties.

X-ray analysis of SsADH is currently being carried out at the Chemistry Department at the University of Naples. Unfortunately, the presence of twinning prevents structural work; thus, to study the microgravity effect on the twinning and to obtain crystals suitable for an X-ray analysis, crystallization of SsADH complexed with NADH was carried out in space.

METHOD: All experiments were carried out by the vapor diffusion method in the APCF facility, using 8 ml hanging drop reactors. Since the optical properties of the reactors prevent visual inspection by microscope, crystals were examined only after removal from the reactors. All operations were conducted at 20 ˚C.

Pre-flight tests were carried out in these reactors to optimize the crystallization conditions. Particular attention was focused on the duration of the whole crystallization process because the reproducibility of this parameter was critical. The dilution ratio was always 2:3 (instead of the usual 1:1) to reduce the crystallization time. Space and ground-control duplicate experiments were carried out in six reactors.

Post-flight analysis of the space-grown crystals was performed using synchrotron radiation on the EMBL beam line X11 at the storage ring DORIS (DESY Hamburg).



ADVANCED PROTEIN CRYSTALLIZATION FACILITY INVESTIGATIONS

Microgravity Crystallization of *Sulfolobus Solfataricus* Alcohol Dehydrogenase (continued)

RESULTS: Typically, crystallization trials using a 6-12 mg/mL solution of the enzyme in Tris·HCl buffer (0.05-0.15 M), pH 8.4, NADH 1 mM, with 2-methyl-2,4-pentane-diol, PMD, as precipitating agent in the concentration range 44-50% (v/v), produce crystals large enough to diffract to 3.0 Å resolution. Usually, crystals grow in size from 400 to 800 μm, within 14 to 30 days. The launch was delayed by 23 days. The reactors were not refilled because the protein is usually very stable, and a batch-dependent variation of the optimal crystallization conditions is usually observed. Space experiments have been carried out changing protein concentration from 8 to 10 mg/mL and MPD concentration from 46 to 48% (v/v). SsADH crystals were obtained in only two duplicates out of six reactors, where the protein and the precipitant concentrations were highest; this was true for both ground and space experiments. The average size was about 100 μm. The crystal habit also presented some irregularities. Despite the small size, two of these crystals were mounted in a Lindmann capillary and were exposed to the synchrotron radiation at DESY. The diffraction power was very poor, preventing any further analysis. The protein, although very stable, occasionally may undergo slight degradation after a long time. This might have occurred during the long delay before the launch, resulting in small-sized crystals.

PRELIMINARY CONCLUSIONS: Crystals formed under the same conditions in space and on Earth provided evidence that the reproducibility of results in microgravity was the same as that on Earth, at least for this protein. This compares with several (but not all) cases cited in the literature. Nonetheless, the purpose of obtaining good-quality crystals was not answered in this mission. This warrants further experiments in space, considering that results gained with a limited number of samples are not sufficient to draw significant conclusions.

ASTROCULTURE™

PIs: **R.J. Bula and T.W. Tibbitts**

AFFILIATION: University of Wisconsin, Madison, Wisconsin

PURPOSE: The purposes of this experiment were to evaluate the performance in microgravity of a unit for supporting growth of plants and to study how starch accumulation in plants is affected by the microgravity environment.

METHOD: Approximately 36 hours before launch, a flight unit capable of providing the environmental conditions required for plant growth was used to grow the leaf cuttings, which were taken from potato plants that were 41 days old. The crew periodically monitored the status of the environment in the plant chamber and the status of the plant material via a video camera in the top of the plant chamber. Post-flight analyses included determination of the size and weight, as well as the chemical, enzyme, and anatomical characteristics of the tubers.

RESULTS:

I. The ASTROCULTURE™ flight unit, a totally enclosed chamber, provided the environment required to support plant growth in microgravity during the 16-day mission. This was the first time plant material has been grown in microgravity in a totally enclosed controlled environment chamber; thus, any unique plant responses can be attributed to the effects of microgravity and not confounded with inadequate control of the other environmental parameters.

II. The ability to downlink video images of the plant material during the mission provided scientists with valuable information as to the status of the plant material. Previously, the scientist had no way of assessing the progress of the microgravity experiment. This capability was incorporated into the ASTROCULTURE™ flight unit.

III. The potato leaf cuttings withstood launch conditions without any appreciable disturbances or problems as evidenced by comparison of the video images taken on the ground before launch with those downlinked on day 1 of the USML-2 mission. Video images showed that the leaf cuttings maintained their vigor and turgidity during the first 12 days in microgravity. They also maintained active rates of photosynthesis and respiration as evidenced by changes in the carbon dioxide concentrations of the air in the plant growth chamber during the periods when the lights were on (the period when photosynthesis occurred) and when they were off (the period when photosynthesis did not occur). Rates of photosynthesis and respiration decreased markedly as the leaf cuttings senesced during the last 4 days of the mission.

IV. The potato leaf cuttings produced tubers (potatoes) that averaged 1.40 grams, fresh weight, and had an average diameter of 1.5 cm. Cuttings taken from potato plants similar to those used for the flight experiments and grown in an ASTROCULTURE™ flight unit maintained in a terrestrial growth room under environmental conditions that simulated those of the *Columbia* middeck area produced tubers that averaged 1.51 grams, fresh weight, and had an average diameter of 1.5 cm. Figure 1 shows the

tubers developed by the leaf cuttings during the USML-2 mission. As a comparison, Figure 2 shows the tubers developed by the leaf cuttings during the 16-day ground-control experiment.

V. Immediately after recovery of the flight unit, the plant material was removed and preserved for biochemical and anatomical analyses. The biochemical analyses are being performed by Dr. Brown in his laboratories at the Kennedy Space Center. The anatomical analyses are being performed by Dr. Croxdale in her laboratories at the University of Wisconsin-Madison.

VI. Providing a remote site for monitoring the mission activities was a very important aspect of the USML-2 mission. The remote site provided the capability to involve more scientists in the ongoing mission activities, thereby increasing the science that will be achieved by the USML-2 mission and providing useful lessons learned for future missions.

PRELIMINARY CONCLUSIONS: Results of the ASTROCULTURE™ experiment on the USML-2 mission clearly demonstrate that potato plants can produce tubers (energy storage organs) in a microgravity environment. The effects of microgravity on the composition and structure of the potato material will be defined upon completion of the biochemical and anatomical analyses.

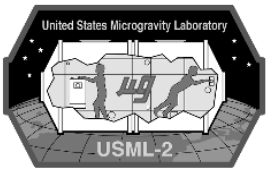
Figure 1: Photograph of the tubers produced on the potato leaf cuttings during the 16-day USML-2 mission.

Figure 2: Photograph of the tubers produced by the potato leaf cuttings during the 16-day ground-control experiment.

The ASTROCULTURE™ team would like to express their thanks and gratitude to all those involved in the USML-2 mission for the support they have given the experiment. A special thanks to the crew of STS-73 for their efforts that have contributed so much toward the success of the USML-2 mission.

They also acknowledge the financial support provided by NASA's Space Processing Division, Code XP, for the development of the ASTROCULTURE™ flight unit and by NASA's Life and Biomedical Sciences and Applications Division, Code UL, for the plant science aspects of this experiment. In addition, they acknowledge the financial and engineering support provided by Quantum Devices, Inc., as an industry partner, in the design and development of the ASTROCULTURE™ flight unit.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS (CGBA)



The Commercial Generic Bioprocessing Apparatus (CGBA) is a multi-purpose facility that can help answer important questions about the exploitable commercial relationships between gravity and biology. This unique facility allows scientists to study biological processes in samples ranging from molecules to small organisms. The CGBA payload replaces a standard middeck locker, which can be flown in the Space Shuttle middeck, Spacelab, or in Spacehab. Samples are contained in a Fluid Processing Apparatus (FPA), a device that is essentially a "microgravity test tube." An FPA is a multi-chambered glass barrel that allows sequential mixing of three fluids. Eight FPAs are housed in a Group Activation Pack (GAP). The CGBA locker provides a uniformly temperature-controlled (37 ˚C) volume for nine GAPs, data acquisition and control electronics, and optical density measurement capabilities (565 nm) for up to eight FPAs at a time. GAPs also can be stored at ambient temperature in middeck lockers or in the Spacelab module. The CGBA locker and its samples can be loaded as late as 18 hours before launch to maximize viability of the biological samples.

Bioprocessing reactions can be initiated using predetermined mixing protocols. Multiple-step reactions involving sequential mixing of fluids are possible for phased processing. Simple optical monitoring of turbidity changes is possible. This capability is a major innovation in the study of biological processes in space. A crewmember can activate experiments by turning a crank on the GAP, thereby initiating the first fluid mixing process. Later in a mission, experiments can be terminated in a similar fashion. Some samples can be monitored for brief periods repeatedly throughout the mission. Both data taken on orbit and the returned samples provide the basis for experimental analyses.

On USML-2, the CGBA supported 23 separate investigations that occupied 9 incubated GAPs and 24 ambient GAPs, for a total of 264 FPAs. One hundred forty-four FPAs were prepared for launch approximately 2 months before the original launch date. These samples were stowed in the Spacelab module under ambient temperature conditions until launch. The remaining 120 FPAs were prepared and turned over for integration into Columbia's middeck approximately 24 hours before launch. Final preparation of late-access samples for the launch was done as planned without significant difficulty; however, several experiments required the use of backup fluids because of the numerous launch delays.

Following launch, payload operations were supported around the clock by BioServe personnel in the Life Science Support Facility (Hangar L) at Kennedy Space Center. Backup support was provided by BioServe personnel at the remote site at the University of Colorado in Boulder. Information and data obtained realtime by on-call BioServe personnel were disseminated to appropriate investigators at their respective test sites. Using such information, investigators were able to perform simultaneous matched ground controls and to make recommendations for changes in crew operations or timelines to improve the science return from their investigations.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS

From both an operations and science return perspective, the payload was successful. All but one GAP were activated and terminated according to the schedules established by the investigators and mission support personnel. For one GAP, which was inadvertently initiated too early, samples still were obtained with only a slight loss of science. Minor anomalies did occur during operations of a few FPAs, but there were no significant overall impacts. The science return was compromised in 32 FPAs, or 12% of the total number. Of these 32 FPAs, 24 samples were impacted because of the combination of early stowage and the numerous launch delays. Eight sample losses were attributable to incomplete activations of a time-critical experiment. The remaining 232 FPAs were operated successfully to yield data and samples for analysis.

During the mission, 16 of the FPAs were transferred to the optical density measuring device within the CGBA for collection of turbidity data. These data provided an indication of the rate at which the biological processes occurred, which could be compared with ground controls upon completion of the mission. Video and photography operations were performed for three experiments over three sessions equally spaced throughout the mission. Photographs have not been received as of this date. Video recordings have been reviewed by the PIs for important data regarding the behavior of samples in microgravity.

Within 3 hours of landing at KSC, the CGBA locker and the six GAPs located in the ambient middeck locker were removed from the orbiter and transported to Hangar L for deintegration. The 18 GAPs located in the Spacelab module were removed and transported to Hangar L for deintegration at approximately 6 hours after landing. Experiments requiring timely post-flight processing (such as fixation, photography, and behavioral gravity-related studies) were performed by PIs at Hangar L. All samples were photographed and packaged for return to the various PI laboratories for analysis.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Plasmin Degradation of Fibrin Clots in Microgravity

PIs: T. Bateman and C. Nunes

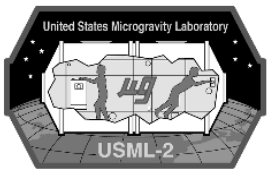
AFFILIATION: Colorado University/BioServe Space Technologies, Boulder, Colorado

PURPOSE: Previous experiments conducted on the Space Shuttle indicated that increased enzymatic activity occurs in microgravity. This experiment was a follow-up study, similar to experiments flown on STS-63 and STS-69. This experiment examined plasmin degradation of fibrin clots at ambient temperatures. The USML-2 studies provided in-flight optical density data and extended the results to include physiological temperatures.

METHOD: This investigation used 6 FPAs at 37 ˚C, 4 with optical density monitoring. Matched flight and ground fibrin clots were prepared on the ground approximately 30 hours before flight, 6 hours before payload turnover. The enzyme, plasmin, was added in microgravity. The degradation process was terminated approximately 3 days into the flight via a 2% GTA solution. The remaining fibrin clot was then weighed upon return to Earth. A second set of 4 monitored FPAs on STS-73 examined the clot assembly and degradation kinetics over an 11-day period.

RESULTS: Initial results suggest that the flight samples were more degraded than ground samples. The optical density data from STS-73 indicated that the lag period for assembly is the same for ground and flight samples.

PRELIMINARY CONCLUSIONS: These preliminary results are consistent with the prior results for fibrin degradation at ambient temperatures. The reason for this increase in plasmin activity is unknown. Future studies will attempt to characterize this increased enzymatic activity via a more detailed chemical analysis.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Development, Growth, and Activation of Bone Marrow Macrophages-Phase II

PI: K. Chapes

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: Bone marrow macrophage development is inhibited *in vivo* by spaceflight or by antiorthostatic suspension. Inhibition of macrophage development and function can have severe consequences on health and immunological function. Experiments on STS-57, -60, -62, and -63 found that bone marrow macrophage differentiation in a liquid culture was significantly inhibited by spaceflight; however, growth was significantly enhanced. Furthermore, experiments from STS-69 demonstrated that bone marrow macrophage colony formation was enhanced during spaceflight.

In addition, experiments on STS-37 and STS-43 found that spaceflight altered bone marrow macrophage secretion of cytokines. The USML-2 experiments were designed to expand on these studies. Investigators tested whether the transcription of receptor proteins or cytokines are altered by spaceflight. They also attempted to confirm that spaceflight-enhanced macrophage colony formation in an agar culture system was comparable to ground controls.

METHODS:

Experiment 1: The continuous bone marrow macrophage cell line B6MP102 was used in these experiments. B6MP102 cells were attached to cytodex 3 beads and were cultured in 2.8 ml of Dulbecco's modified Eagle's Medium. Cells were exposed to fresh medium or medium including 12.5 μg/ml lipopolysaccharide. After 18 hours the experiment was terminated by the addition of guanidinium HCL. RNA was to be purified from the samples after FPA recovery. Cells were analyzed for their transcription of TNF and other cytokine genes.

Experiment 2: Freshly harvested bone marrow cells were isolated from the marrow of C3HeB/FeJ mice. Marrow cells were resuspended in 1.5 ml of soft agar-containing medium and macrophage colony-stimulating factor, and the FPAs were plugged with gas-exchange septa. The FPAs were gassed in an 8% CO_2 incubator for more than 8 hours, and the FPAs were sealed with conventional septa leaving more than 5 ml of an air atmosphere with an 8% CO_2 concentration. The FPAs were stored at 37 °C during flight, and colony formation was quantitated upon FPA recovery.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS

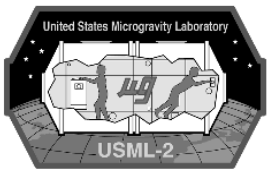
Development, Growth, and Activation of Bone Marrow Macrophages-Phase II (continued)

RESULTS:

Experiment 1: The FPAs were stored in an ambient temperature gap during the flight of STS-73. This gap was terminated at the designated time, the samples were recovered, and RNA was purified from the samples. Several ground control FPAs were not terminated correctly; therefore, there was not enough control RNA for investigators to make valid comparisons. Alternative data analyses are planned for these samples.

Experiment 2: The FPAs demonstrated good colony formation using the soft-agar technique. The experiment provided 76 $\pm$ 6 (mean $\pm$ SEM) colonies per FPA flown in space as compared to 98 $\pm$ 6 colonies in ground controls maintained in Florida. In addition, cells in soft agar secreted 2,280 $\pm$ 279 ng/ml of soft agar when flown in space, compared to 1,730 $\pm$ 390 ng/ml soft agar for the Florida ground controls.

PRELIMINARY CONCLUSIONS: These experiments confirmed the data from STS-69, where it was demonstrated that soft-agar bone marrow cultures analogous to those done in conventional lab settings could be done; however, results also showed that marrow colony formation was depressed by spaceflight. This contrasts with the data obtained from STS-69. The trend for enhanced IL-6 secretion also contrasts with the results of STS-69. However, the methodology to assay IL-6 was changed from that used to assay for IL-6 in STS-69. It is possible that the contrasting results between STS-69 and STS-73 are caused by the differences in flight lengths. It is possible that a 16-day flight allowed for overgrowth and death of colonies that would have been quantitated at day 8 (the length of STS-69 flight), and the 16-day flight was more satisfactory to slower forming colonies. Additional ground-based experiments are planned to test this hypothesis. STS-73 was also plagued by several delays. Unfortunately, the investigator team's poorest bone marrow cell preparation eventually flew. They think that earlier loads, which were aboard the scrubbed launches, were better cultures as assessed by the eventual colony formation that was seen; therefore, the quantity of the cell preparation may have influenced the final data.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Effects of Space on Biochemical Reaction Kinetics

PI: K. Chapes

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: The purpose of this experiment was to obtain quantitative data to determine the reliability of biochemical reactions that occur in spaceflight. As BioServe enters into more collaborations with commercial sponsors, there is a need to substantiate to potential partners that certain biochemical assays that are used to quantify biological processes on Earth still can be used reliably in a microgravity environment. For example, the use of peroxidase enzyme-conjugated probes and cognate substrates are used to quantitate several kinds of assays. The cell metabolic products, such as nitrite and peroxidase assays, were quantitated during spaceflight, and those data were compared to controls performed on Earth. These data would have been useful during discussions with potential commercial partners when they had questions about the reliability of quantitative assays done during spaceflight.

METHODS:

Fluids required:

- 1% sulfanilamide
- 0.17% napthylethylene diamine dichloride
- 2.5% H_3PO_4
- 100 mM $NaNO_3$
- 1 mg/ml biotin peroxidase
- 300 mg/ml 4 chlor 1 napthol
- 99% methanol
- 20 mM tris base
- 500 mM NaCl
- 3% H_2O_2

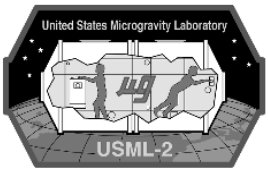
Special requirements: Continuous spectrophotometer readings at 565 nm.

Various concentrations of the Griess reagent and peroxidase were loaded into the eight FPAs during the early load period. At the appropriate time during STS-73, these FPAs were to have been activated and spectrophotometric data collected.

RESULTS: These FPAs were not activated according to the designated protocol; therefore, the data for the peroxidase experiment are not usable. The data for the detection of nitrite and nitrate have yet to be forwarded to Kansas State University from Colorado. There is a possibility that these FPAs were not inserted into the spectrophotometer according to the written protocol; therefore, the data collected are currently questionable.

PRELIMINARY CONCLUSIONS: No conclusions have been reached to date. The data possibly were compromised because of inadvertent activation at the wrong time.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS



Viral Infection of Mammalian Cells in Microgravity

PI: R. Consigli

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: The purpose of this experiment on STS-73 was to determine if microgravity affects the ability of a virus to infect mammalian cells.

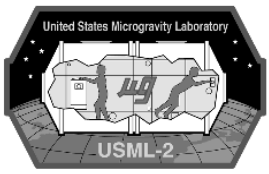
METHODS: The virus used in these experiments was polyomavirus, a small DNA virus that has the ability to infect murine (mouse) cultured 3T6 cells. The experimental approach was two-fold:

I. Cells that were previously cultured on coverslips were inserted into the FPA, Chamber A.

II. At a specific time, the virus that was in Chamber B, containing virus and S^{35} methionine, was activated at the appropriate time and was terminated by exposing the cells to Triton X-100 (Chamber C) at 1-, 2-, and 3-day intervals. This experiment was analyzed by isolating the viral proteins by immunoprecipitation and by separating the proteins by SDS-PAGE. The viral proteins were further analyzed by Western Blot and fluorography. In both cases, 3T6 cells were cultured in Chamber A 24 hours before transport to the NASA facility.

RESULTS: Because of the numerous launch delays STS-73 experienced, the resulting multiple sample reloads, and the 16-day flight, it was found that the cultured cells underwent some adverse conditions, possibly as a result of depletion of nutrients. The experiment, which used the infection of cultured cells on coverslips, indicated that infection was proceeding in microgravity; however, it was difficult to determine if infection was improved or reduced in microgravity because most of the cells had detached from the coverslip surface. The experiment, which used S^{35} methionine incorporation and immunoprecipitation to isolate the viral proteins selectively and analyze them by SDS-PAGE flourography, did not show any incorporation of radio-labeled amino acid into the viral proteins. Lack of radio-labeled incorporation into viral proteins also was found with the ground-based experiment. Investigators believe the cultured cells did not remain viable because of the flight delays.

PRELIMINARY CONCLUSIONS: Experiment results indicate that microgravity will allow viral infection of cells; however, at this time, it is difficult to determine whether microgravity improved or reduced infection. Investigators feel that this is a very important experiment and should be repeated under more stringent controlled conditions. A shorter flight, which would allow cells to be maintained more appropriately, would improve results, as would a reduced methionine concentration in the media, which would allow maximum incorporation of the radio-labeled amino acid into newly synthesized viral proteins.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Effects of Microgravity and Clinorotation on Ethylene Production in Mutants of *Arabidopsis* with Altered Starch Regulation

PIs: G. Gallegos and J. Guikema

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

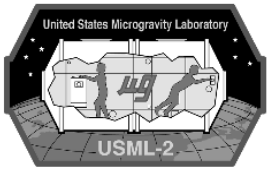
PURPOSE: Plants having amylopasts containing less starch exhibit a corresponding delay in gravitropic response. Growing sweet clover (*Melilotus alba* L.) and soybean seedlings in the altered gravity condition of a slowly rotating clinostat has resulted in enhanced ethylene production. Additionally, investigators have suggested that this is an IAA-induced response resulting from continuous gravistimulation rather than a result of the simulation of a microgravity condition. If so, they expect that plants deficient in starch accumulation in amyloplasts may produce less ethylene when grown on a clinostat.

METHOD: To test this hypothesis, *Arabidopsis thaliana* was grown in the Fluid Processing Apparatus. Stationary plants were compared with clinorotated plants and those grown in microgravity aboard *Endeavour* during STS-69 in September 1995. In addition to the wild-type, three mutants with lesions were used, resulting in altered starch regulation. Mutants TC7 and TL25 are deficient in starch biosynthesis as a result of being deficient in the activity of amyloplast phosphoglucomutase and ADP-glucose pyrophosphorylase, respectively. Mutant TC265 is deficient in starch degradation. Seeds of the wild-type and the three mutants were germinated and grown for three days in the FPA before being fixed. Upon recovery, gas samples were quantified for carbon dioxide and ethylene by a Hewlett Packard 5880 Gas Chromatograph with the Thermal Conductivity Detector in tandem with the Flame Ionization Detector (GC/TCD/FID).

RESULTS: Ethylene production was very high in all three mutants for all four treatment groups. It was significantly greater in all three mutants when compared to that of the wild-type in the static and in the vertical and horizontal clinorotation treatment groups; however, the horizontally clinorotated wild-type produced a greater amount of ethylene than when vertically clinorotated or static. Static and vertical horizontal groups were the same in their ethylene production. The wild-type produced a significantly greater amount of ethylene in microgravity than in the ground-based static treatment. Investigators have seen this in soybeans but not in *Melilotus*. This response may be species-specific. In the microgravity treatment group, the wild-type produced about as much ethylene as did the three mutants.

Effects of Microgravity and Clinorotation on Ethylene Production in Mutants of *Arabidopsis* with Altered Starch Regulation (continued)

PRELIMINARY CONCLUSIONS: Mutants TC7 and TL25 have a severely reduced ability to store carbon in the form of starch. This is especially true for the plastid since the TC7 lesion is the inactivity in the plastid isozyme of phosphoglucomutase, the enzyme responsible for the conversion of glucose-6-phosphate to glucose-1-phosphate. Is the amyloplasts' inability to form dense starch grains, therefore severely reducing their statolith function in columella cells of the root cap, the reason for the significant increase in ethylene production by TC7 and TL25? Perhaps the diversion of carbon flux through glycolysis and the oxidative pentose phosphate pathway by mass action is a more likely cause of the increase in ethylene production. Mutant TC265 does not have a problem storing carbon as starch. To the contrary, TC265 is severely inhibited in starch degradation. This too, as with TC7 and TL25, is an alteration in carbon flux and in carbon partitioning. Also, as observed with TC7 and TL25, ethylene production was significantly increased in TC265. Does the developing seedling perceive these drastic alterations in carbon flux and carbon partitioning as stress? It appears the increased ethylene production in mutants TC7, TL25, and TC265 was stress-induced; additional mutants will have to be used to more fully address the hypothesis.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Starchless *Arabidopsis* Mutant

PI: E. Hilaire

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

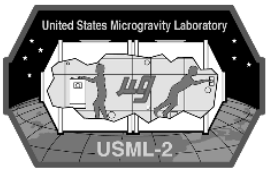
PURPOSE: In higher plants, columella cells of the root cap are proposed to be sites of gravity perception. These cells contain starch-filled plastids, which sediment in the direction of the gravity vector and initiate the gravity signal transduction cascade. It was of interest to study plastid location in the columella cell, when both the gravity vector and the starch content were altered.

METHOD: In this study, starchless *Arabidopsis thaliana* mutant seedlings (TC7) were germinated and grown for 72 hours in the Fluid Processing Apparatus under 4 gravitational treatments: stationary; slow clinorotation (2 rpm); stationary, followed by centrifuge (20g for 5 min); and microgravity of spaceflight. Seedlings were fixed under the same gravitational treatment, and the root tips were processed for electron microscopy. Location of the starchless plastids in the columella cells was studied by computer image analysis from longitudinal sections.

RESULTS: In the stationary treatment, starchless plastids were equally located in the mid- and distal third of the columella cells. When stationary roots were further treated by centrifuge at 20g for 5 min, most of the plastids were found in the distal third of the cells. Plastids from the flight samples were mostly distributed in the proximal third, whereas the clinorotated seedlings were characterized by plastids located mostly in the middle third of the columella cells.

PRELIMINARY CONCLUSIONS: These data suggest that the decrease in plastid density resulting from a lack of starch does not prevent redistribution of plastids caused by gravity treatments. This study indicates that plastid distribution is different between clinorotation and microgravity treatment, suggesting that, on a cellular level, clinorotation is a poor simulation of microgravity.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS



CeReS-Mediated Cell Stabilization

PI: T. Johnson

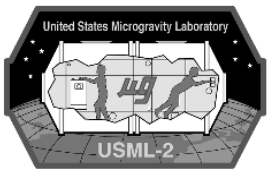
AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: This experiment was designed to follow the successful experiments conducted on STS-69 and to provide independent evidence that the cell cycle arrest, mediated by CeReS-18, could efficiently be reversed in the microgravity environment. In addition, an alternative way to reverse the cell culture arrest was examined.

METHOD: The experiment was conducted essentially as it was aboard STS-69. In addition to testing the reversal by dilution of the CeReS-18 to a non-inhibitory concentration with culture medium, the potential use of increased calcium ion concentrations to induce cell cycle escape also was examined. On STS-73, the incubation time, following the reversal of cell inhibition, was 5 days.

RESULTS: The results from this flight, when the cell cycle arrest was reversed by medium dilution, confirmed the data obtained from STS-69, which showed that growth inhibition could be reversed in the microgravity environment. In addition, the reversal and subsequent growth kinetics once again were comparable to matched ground-based controls. Attempts to reverse cell cycle arrests by raising the medium concentration of calcium ion, however, were more complex. Although the cells successfully escaped from inhibition when fresh medium with elevated calcium ion was introduced to the arrested cell cultures, preliminary analysis suggests that it was not necessarily the calcium ion concentration, but rather the introduction of fresh culture medium, that initiated the reversal.

PRELIMINARY CONCLUSIONS: The potential application of CeReS-18 as an agent for stabilizing cell cultures for future microgravity missions was further strengthened. If calcium ion shifts are to be utilized as a reversal strategy, additional studies will be necessary.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

E. coli Growth and Development

PI: D. Klaus

AFFILIATION: Colorado University/BioServe Space Technologies, Boulder, Colorado

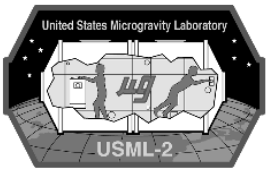
PURPOSE: Similarities between altered growth kinetics in space, presumably caused by weightlessness, and a biotechnology processing method that uses controlled glucose feeding to increase biomass production was studied. This fundamental relationship was explored for potential application to bioprocessing in space. Many bioprocessing techniques rely on keeping cultures suspended without introducing significant shear forces on the fluid. Weightlessness offers the opportunity to study the relative contribution of diffusion in the absence of sedimentation.

METHOD: This experiment used 4 FPAs at 37 °C with Optical Density monitoring. Matched flight and ground *E. coli* suspension cultures, in a glucose-based synthetic medium, were loaded, initiated, and monitored (turbidity) simultaneously for comparison of growth characteristics. Population counts were performed post-flight for the fully saturated cultures. Optical density data were collected in realtime to determine times of transition between the lag, exponential, and stationary phases.

RESULTS: A statistically significant increase ($p<0.001$, t-test) of 29% was observed in final cell density in space (6.7×10^8 cells/ml) relative to the matched ground controls (5.2×10^8 cells/ml). The dynamics of the growth curve generated from the turbidity measurements are currently being analyzed.

PRELIMINARY CONCLUSIONS: The finding of a net increase in cell density in space is in agreement with previous data similarly obtained and is generally corroborated in the literature. An understanding of the underlying mechanisms associated with the observed changes remains, however, for the most part, elusive. By studying the kinetics of the growth curve, it is hoped that the observed changes can be categorized and subsequently utilized in novel space bioprocessing applications.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS



Pre-metatarsal Development

PIs: B. Klement and B. Spooner

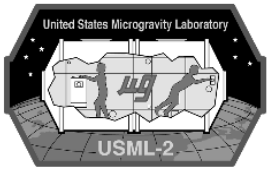
AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: The experiments performed on this flight were similar to experiments performed on previous flights. Investigators were interested in examining growth, mineralization, and development of embryonic bone rudiments during culture in microgravity.

METHOD: Pre-metatarsal tissue from embryos at 13 days of gestation was removed, placed on gas exchange inserts, and covered with Matrigel. Chambers A, B, and C contained nutrient BGJ_b culture medium. Chambers B and C contained ^{45}Ca to monitor Ca incorporation into the potentially mineralized extracellular matrix. All pre-metatarsals were fixed after landing. The length of the ground-control pre-metatarsals and flight pre-metatarsals were measured.

RESULTS: Preliminary findings show that the average length of the ground controls was 1180.8 μm, and the average length of the flight pre-metatarsals was 1128.5 μm.

PRELIMINARY CONCLUSIONS: Additional ground-control experiments are currently being conducted. Analysis of mineralization and the extent of differentiation are still under investigation.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Effects of Microgravity on Auxin-Inducible Gene Expression in *Arabidopsis*

PI: Y. Li

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

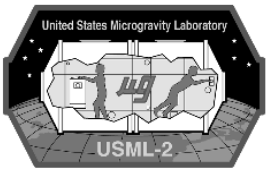
PURPOSE: The purpose of this experiment was to investigate the effects of microgravity on auxin accumulation and the expression of auxin-inducible genes in higher plants.

METHOD: *Arabidopsis* was germinated and grown in space aboard STS-73. The mRNA will be isolated from the space-grown seedlings to confirm whether the expression of auxin-inducible SAUR and GH3 gene were altered in space.

RESULTS: Since the plant materials harvested from STS-73 are not sufficient to perform Northern Blot analysis, the tissues have been stored and will be processed later.

PRELIMINARY CONCLUSIONS: No conclusions can be reached at this time.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS



Effects of Microgravity on the Growth and Development of *Pseudomonas aeruginosa* Biofilms

PI: B. Manfredi

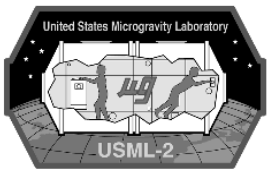
AFFILIATION: University of Colorado/BioServe Space Technologies, Boulder, Colorado

PURPOSE: Bacteria secrete extracellular polymeric substances that adhere to surfaces and create a matrix of organic materials called biofilm. The growth of *Pseudomonas aeruginosa* biofilms was investigated on USML-2. There are many materials present in spacecraft water recycling systems, and the susceptibility of these surfaces to form biofilms in low gravity should be determined. This research contributes to efforts at improving the resistance of public water recycling surfaces to biofilm formation.

METHOD: Eight flight and eight simultaneous static and tumbled ground samples of *Pseudomonas aeruginosa* were grown in FPAs at ambient temperature for the duration of the flight. Bacteria (1 ml) were initiated into 8 ml trypticase soy broth, where a variety of polymeric and other surfaces, hydrophobic and hydrophilic, were submersed. Following experimentation, total cell counts of bacteria were determined using epifluorescent microscopy, and the biofilm was analyzed using a scanning electron microscope (SEM).

RESULTS: The SEM analysis revealed a bacterial contaminant, a coccus species, and possibly a mold. This contamination probably resulted from reloading the bacteria in the original FPAs twice as a result of launch delays. The number of *Pseudomonas aeruginosa* attached cells and size of bacterial colonies on all surfaces was approximately 90% smaller than those from STS-69.

PRELIMINARY CONCLUSIONS: Because of the contamination, no conclusion can be drawn from this flight, and the experiment must be repeated.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Brine Shrimp Development in Space

PIs: A. Paulsen and B.S. Spooner

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: Brine shrimp are small crustaceans of major commercial importance; they are used as food in aquaculture, as a protein source for humans, and as human physiology models for toxicology and sensitivity to anesthetics testing. Their availability as developmentally arrested cysts makes them important systems for studying animal development during spaceflight.

METHOD: Cysts in Chamber A of the FPAs were developmentally reactivated on orbit by introducting a nutrient medium from Chamber B. Development was then terminated after 1, 3, 4, 8, or 10 days by introduction of a fixative from Chamber C.

RESULTS: From the data obtained on this flight, confirmation of previous findings was achieved: developing brine shrimp are going through sequential instar stages. Three discouraging events occurred on the USML-2 flight of the project. The percentage of cysts that hatched compared to earlier flights (25% on this flight compared to 40-45% on previous flights) decreased. A higher percentage of the brine shrimp were observed in various stages of death and decay than in earlier missions, which made it difficult to analyze the amount of development achieved. Results from earlier flights suggested that brine shrimp that had been developing for longer time periods were undergoing sexual maturation, but no evidence of this was seen in data from this flight. The reasons for these flight differences are unknown, and additional experiments will be performed to investigate them.

PRELIMINARY CONCLUSIONS: In previous experiments, brine shrimp that hatched during spaceflight displayed accelerated development compared to ground controls. The data obtained from this flight do not contradict earlier conclusions about brine shrimp growth and development, but no significant new findings were revealed.

COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Effect of Gravitational Unloading on Plant Gravity Response

PI: J. Smith

AFFILIATION: Colorado University/BioServe Space Technologies, Boulder, Colorado

PURPOSE: Investigators have previously observed larger-than-control amyloplasts (gravity sensors) in the root tips of clover after germination and growth for 3 days aboard the Space Shuttle (STS-60). The aim of this experiment was to test other candidate species, corn, clover, and *Arabidopsis*, for similar gravitational effects on their gravity sensors and to test for a change in plant sensitivity to gravity as a result of larger-than-control amyloplasts.

METHOD: Seeds of three species, corn, clover, and *Arabidopsis* were placed on filter paper in 32 FPAs. On orbit, the seeds were soaked in water (corn and clover) or nutrient solution (*Arabidopsis*). Corn grew for 5 days in darkness; clover grew 4 days in darkness; and *Arabidopsis* grew 4 days under room lighting conditions. All plants were returned to Earth in a viable state, subsequently photographed, and, where applicable, the sensitivity of the plants to gravity was measured.

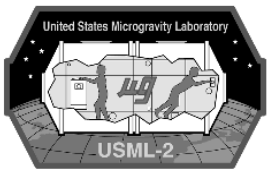
RESULTS:

Corn: Initiation error allowed half of the corn to grow for 16 days, which was too long.

Clover: The 4-day growth duration for clover in the GE-FPA allowed for greatly increased plant length over previous studies performed in standard FPAs.

Arabidopsis: No flight plants germinated; however, ground controls germinated over 90% of their seeds. Temperature fluctuations aboard the Shuttle may have been responsible.

PRELIMINARY CONCLUSIONS: Analyses are being conducted at this time.



COMMERCIAL GENERIC BIOPROCESSING APPARATUS

Effects of Microgravity on the Legume-*Rhizobium* Nodulation Process

PI: P. Wong

AFFILIATION: Kansas State University/BioServe Space Technologies, Manhattan, Kansas

PURPOSE: Legumes such as soybeans, cowpeas, and clover play an important role on Earth and, in the future, on a permanently manned space station. Legumes provide protein-rich food and form nitrogen-fixing root nodules. As a result of this symbiotic relationship with the bacterium *Rhizobium*, legumes can be grown without the use of nitrogen fertilizer. The goals for this experiment were to investigate whether the nodulation process can occur in microgravity and whether gravity plays a role in the recognition process between the legume and the *Rhizobium*.

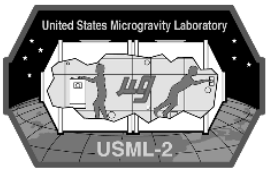
METHOD: Clover plants are small enough to survive for at least 7 days in the FPA. Two strains of *Rhizobium*, TA1 and T24, were used to nodulate the clover plants. However, strain T24 produces a toxin that specifically kills strain TA1. As a result, after 5 days, strain TA1 was inoculated with T24. The first batch of the nodules was induced by TA1, which can be identified by antibodies and genetic markers. The nodules developed later were induced by T24, which also can be identified.

On 24 October 1995, at 10:48 p.m., clover seeds in the FPA were watered and inoculated with strain TA1. The seedlings developed and grew. On 3 November, 1995, at 6:16 p.m., the clover seedlings were inoculated with strain T24. Upon return to Earth, the seedlings were planted and examined for nodules induced by the two strains.

RESULTS: The clover plants were examined for root nodules. Each plant, whether experimental or ground control, had two to three nodules. The nodules were crushed, and the *Rhizobium* was cultured in a yeast extract-marmitol medium. The strain of the *Rhizobium* was identified by specific antibodies. All the nodules were induced by strain TA1.

PRELIMINARY CONCLUSIONS: If the nodulation process occurs in microgravity, the nodules induced by strain TA1 should be found. If not, only nodules induced by T24 will be found. All nodules were induced by strain TA1, suggesting that the early steps of the legume nodulation process can occur under microgravity conditions; however, it is not known whether the specificity of the interaction between legume and *Rhizobium* can be altered under microgravity. The goal for future experiments will be to test if *Rhizobium* strains that do not nodulate plants such as wheat and rice can nodulate them under microgravity.

CRYSTAL GROWTH FURNACE



Orbital Processing of High-Quality Cadmium Zinc Telluride (CdZnTe) Compound Semiconductors

PI: D. Larson

AFFILIATION: The State University of New York at Stony Brook, New York

PURPOSE: This program is a coordinated research effort involving both orbital and ground-based research. The technical objective of this program is to investigate quantitatively the gravitational influences (hydrostatic and buoyant) on the growth and quality of alloyed CdTe compound semiconductors. Specifically, the seeded, modified Bridgman-Stockbarger crystal growth technique is being investigated.

This experiment built upon a substantial 1-g and microgravity technical foundation that was established in the course of an investigation conducted on STS-50/USML-1. The USML-1 program confirmed that diffusion-controlled crystal growth was achieved during orbital processing of CdZnTe, as predicted by investigators' fluid flow and solute redistribution model, with concomitant improvement in chemical homogeneity, both longitudinally and radially. In addition, dramatic improvement in structural quality was demonstrated, particularly in those regions that solidified without wall contact, as hypothesized in the experiment proposal. In these regions, commonly referred to as "dewetted," twinning was virtually eliminated, though it is pervasive terrestrially. This was not anticipated. Further, the (111) [110] dislocation density was reduced by almost three orders of magnitude, to an unprecedented value of approximately 800 epd. The extremely low dislocation density virtually eliminated all dislocation substructure. The USML-2 program further investigated the origins of these results, both qualitatively and quantitatively, in the primary experiment. In the secondary experiment, investigators attempted to exploit a unique ampoule design to minimize wall contact during the crystal growth processing and post-solidification heat treatment.

METHOD: Pre-flight operations focused on developing flight ampoules and sample ampoule cartridge assemblies, optimizing the thermal profiles to be used during the flight experiments, and establishing a 1-g comparative baseline for each ampoule geometry. In addition, detailed analysis of the USML-1 flight samples was continued and the high-fidelity thermal, thermo-solutal, and thermo-mechanical process models were refined.

Two flight experiments were conducted successfully aboard USML-2 in the Crystal Growth Furnace (CGF), using the seeded Bridgman-Stockbarger technique. Both samples were processed with a hot end temperature of 1175 °C and a cold end temperature of 980 °C, with a thermal gradient of 36 °C/cm at the solidification temperature of 1095 °C.

The primary sample had a growth period of approximately 70.5 hours, during which time 110 mm of crystal was grown at 1.6 mm/hr. The

Orbital Processing of High-Quality Cadmium Zinc Telluride (CdZnTe) Compound Semiconductors (continued)

secondary sample had a growth period of 31.5 hours, during which 52 mm of crystal was grown at 1.6 mm/hr. Both samples were furnace cooled at the maximum rate of cooling from the stopping position.

RESULTS: The two flight samples, the time-temperature-translation records, and the accelerometer data are presently being evaluated. These results will be compared qualitatively and quantitatively with the 1-g ground truth samples, the flight samples processed on USML-1, and the high-fidelity process models. Characterization that uses optical and infrared microscopy, differential chemical etching, FTIR spectroscopy, X-ray radiography, rocking curve and precision lattice parameter mapping, and X-ray synchrotron topography is being conducted.

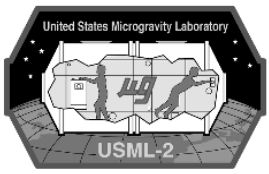
Post-flight radiography showed that the thermocouples shifted less than 1 mm, with a measurement certainty of about 0.5 mm, which was considered to be acceptable. The thermal data, summarized above, were very similar to that experienced on the ground, with the exception of the seeding region of the secondary sample, where the melt-back was greater than experienced on the ground by approximately 5 mm. The sample is being evaluated using the process models.

The radiographs showed that seeding was successfully accomplished in both geometries. It also was shown that the PBN springs worked beautifully in the primary sample, confining the liquid with a stroke of 0.40" as compared to the theoretical stroke of 0.38". This was considered very good agreement and confirmation that the experiment design was sound. The secondary sample was not constrained by springs, and it was found that the initial 11 mm of regrowth was defined by the 6-mm diameter seed holder. This was followed by 20 mm of growth totally without wall contact. The remaining 21 mm of crystal grew with partial wall contact. The balance of the sample was furnace cooled. The successful seeding and the 20 mm of crystal growth without wall contact were considered to be outstanding results.

Analysis of the USML-1 flight samples reported that regions solidified without wall contact were virtually devoid of twins, suggesting that these pervasive terrestrial defects are largely surface nucleated in areas of high stiction. Further, these regions of the USML-1 flight samples showed dramatic reductions in (111)[110] dislocation density, dropping from 800,000 (1-g) to 800 (microgravity) epd. The thermo-mechanical process model suggested that this resulted from thermo-mechanical stress reduction within the flight samples during growth and post-solidification cooling because of the absence of wall contact. Regions of partial wall contact showed defect gradients, with high densities on the wall side and low densities on the free surface side as the model predicted.

Orbital Processing of High-Quality Cadmium Zinc Telluride (CdZnTe) Compound Semiconductors (continued)

PRELIMINARY CONCLUSIONS: Surface X-ray synchrotron topography of the USML-2 flight samples showed that the primary sample, which was forced to maintain wall contact by the restraining springs, was highly strained on the exterior, and twins were common at the surface. This supported the contention that ampoule wall contact during growth and post-solidification processing and concomitant stiction are significant contributors to twin generation. The secondary flight sample's X-ray synchrotron topographs, particularly in the 20-mm section that was solidified without wall contact, were extremely sharp, suggesting very low surface strain and the possibility that this material will reproduce the extremely low defect densities measured in selected regions of the USML-1 flight samples over much greater sample volumes. Once again, in the region without wall contact, the incidence of twin formation was virtually zero. These results, though preliminary, strongly support the conclusions drawn from the USML-1 flight samples and demonstrate that extremely high-quality materials can be grown in space if the unique advantages of orbital processing are exploited in the experiment design.



CRYSTAL GROWTH FURNACE

The Study of Dopant Segregation Behavior During Crystal Growth of GaAs (Gallium Arsenide) in Microgravity

PI: D. Matthiesen

AFFILIATION: Case Western Reserve University, Cleveland, Ohio

PURPOSE: This experiment investigated techniques for uniformly distributing a dopant, selenium, during the growth of gallium arsenide crystals. Specifically, the effect of the thermal profile on the melt/solid interface shape and the corresponding effect on the radial and axial dopant distribution were examined. A secondary objective of these experiments was to test new theories developed from the GaAs crystals on USML-1.

METHOD: Two experiment samples were processed on USML-2. The experiments were designed to minimize natural convection by processing in microgravity and to minimize surface tension driven convection through the use of a spring designed to prevent the formation of a free surface on the molten gallium arsenide. The single-piece crystals were grown using the Liquid Encapsulated Czochralski (LEC) technique (a typical ground-based technique). The crystals were partially melted and then regrown in microgravity using the Bridgman-Stockbarger technique.

The first sample was processed for 67 hours, 45 minutes and included 19 hours of growth at 0.5 microns/sec to grow 3.42 cm and 5 hours of growth at 1.5 microns/sec to grow 2.7 cm.

During the second experiment, the furnace temperature was adjusted in real time via uplinked commands from the Payload Operations Control Center to the furnace. These commands moved the melt/solid interface position toward the hot end of the furnace. The second sample was processed for 50 hours, 10 minutes and included 11 hours of growth at 0.5 microns/sec to grow 1.98 cm and 1 hour, 25 minutes of growth at 5.0 microns/sec to grow 2.6 cm. This sample provides an order of magnitude change in growth rate and reproduces one of the growth rates used during USML-1.

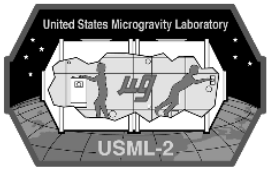
RESULTS: The cartridges containing the samples have been X-rayed at Marshall Space Flight Center. The X-rays were used to measure the position of the 6 thermocouples that were inside the cartridge during processing. These data are essential to correctly interpret the thermal data when they become available. Preliminary data indicate that the desired thermal profiles were achieved. The X-rays show that the crystals are in contact with the container along the length of the crystals and that no voids were formed in the crystals. The absence of voids in these two samples is a result that differs from the results of USML-1 and will be examined further.

CRYSTAL GROWTH FURNACE

The Study of Dopant Segregation Behavior During Crystal Growth of GaAs (Gallium Arsenide) in Microgravity (continued)

The ampoules have been removed from the cartridges. Further characterization is awaiting completion of the ground equivalent tests. The ampoules will be opened in a mass spectrometer to check for any residual gases. The samples will be removed from the ampoule, cut, and polished. Sections of the crystals will be analyzed using an array of characterization methods, including electrical, chemical, and optical techniques. Electrical measurements will include Hall effect and capacitance-voltage techniques. Optical measurements will include quantitative infrared microscopy and Fourier transform infrared spectroscopy. The data from these measurements will be compared to current analytical and computer-model-based theories of crystal growth.

PRELIMINARY CONCLUSIONS: Preliminary results indicate that both crystals were grown successfully and that no free surfaces were formed at the surface of the crystal during the growth process. The absence of voids in either sample indicates that growth rate changes alone were not responsible for the formation of voids seen in the USML-1 samples.



CRYSTAL GROWTH FURNACE

Vapor Transport Crystal Growth of Mercury-Cadmium-Telluride in Microgravity

PI: H. Wiedemeier

AFFILIATION: Rensselaer Polytechnic Institute, Troy, New York

PURPOSE: The primary purpose of this experiment is to understand the initial phase of the process of vapor crystal growth of complex, alloy-type semiconductors. To achieve this goal, crystalline layers of mercury cadmium telluride (HgCdTe) are grown on a (100) oriented cadmium telluride (CdTe) substrate by chemical vapor transport from a single source material using HgI_2 as the transport agent. The specific properties to be determined include the effects of microgravity on the morphology, on the chemical composition, on the structural characteristics and uniformity, and on other properties of the crystalline layer and of the interface. The results of the ground-based and microgravity experiments will be compared to the properties of crystalline layers of this material grown by other techniques to better understand and to improve crystal growth on Earth.

METHOD: The samples were processed in the CGF using chemical vapor transport crystal growth techniques, which do not require temperatures as high as the directional solidification process used on other samples. For this experiment, the hot zone (source region) of the furnace was 595 ˚C, and the cold zone (growth region) was 545 ˚C for deposition. The associated temperature gradient was held steady over the sample.

Optimal experiment conditions (in terms of source material composition, transport agent pressure, and source and growth temperatures) were developed using the same ampoule design. Because of the considerably shorter growth times of the USML-2 experiments relative to those of the USML-1 mission, the growth parameters are even more critical to obtain optimal results. The growth parameters and the ampoule-cartridge assembly were identical for both the ground-based tests in the CGF and the flight experiments.

It should be noted that all ground-based experiments were performed under the vertical, stabilizing orientation. This orientation minimizes gravity-driven convective contributions to the mass transport and crystal growth processes. This will allow any measurable effects of microgravity and of residual convection on the mass transport and growth properties of the HgCdTe - HgI_2 system to be observed through comparison of flight and ground-based samples.

Vapor Transport Crystal Growth of Mercury-Cadmium-Telluride in Microgravity (continued)

RESULTS: Two samples (one primary and one secondary) were processed during the USML-2 mission. The total processing time of the primary sample was 15.5 hours, of which 2.5 hours were growth time. The processing and actual growth times for the secondary sample were 14.5 and 1.5 hours, respectively.

After the return of the samples to Marshall Space Flight Center, X-ray photographs were taken of the ampoule-cartridge assemblies to verify that the thermocouples were in the proper locations along the ampoules. After removal from the cartridge, the mechanical integrity of the fused silica ampoules was confirmed.

Based on visual inspection of the ampoules, the source materials and the sapphire-substrate assemblies were in their proper locations and did not show any structural damage. The surfaces of the source materials in both ampoules showed some recrystallization, which is typical for these procedures and conditions.

The present morphological and compositional analysis of the two samples is based on non-destructive optical microscopy and infrared spectroscopy (IR) measurements.

In both growth experiments, the $Hg_{1-x}Cd_xTe$ deposited on the CdTe substrates is a single crystalline layer throughout the entire area of deposition. The epitaxially grown layer (primary sample) and islands (secondary sample) have very flat surfaces and high spectral reflectivity. The layer of the primary sample appears mirror-smooth, whereas the surfaces of the corresponding ground test layers display a step-terrace, wavy structure. These observations are consistent with and confirm those of the USML-1 experiments. Because of the shorter growth durations employed for the USML-2 experiments, the surface morphology of the secondary sample is different than that observed for the USML-1 experiments.

For the primary USML-2 sample (2.5 hours growth time), the substrate surface is mainly covered by a uniform (100) $Hg_{1-x}Cd_xTe$ layer of the above mentioned flatness and reflectivity. Around the edge of the substrate, the presence of some islands is observed, many of which have partially coalesced. It is important to note that the joining of these islands occurred without the formation of visible grain boundaries. The above observations indicate that the growth time employed for the primary sample is essentially the critical time required for the transition from growth of individual islands to that of a uniform layer under present conditions.

For the secondary USML-2 sample (1.5 hours growth time), the substrate surface is uniformly covered by individual, three-dimensional $Hg_{1-x}Cd_xTe$ islands. The islands have very well developed (100) surfaces of high flatness and reflectivity and other equally smooth crystallographic faces. These observations confirm that the initial island growth is parallel to the (100) substrate surface. Some islands have reached the

Vapor Transport Crystal Growth of Mercury-Cadmium-Telluride in Microgravity (continued)

state of coalescence. The above observations demonstrate that the growth time employed for the secondary sample is below the critical time required for the transition of individual islands to the growth of a uniform layer. The island growth for the secondary sample was expected, based on Ground Control Experiment Laboratory test experiments.

The distinct morphological differences between the two USML-2 samples (layer versus islands) reflect basic differences in the development state of epitaxial growth. Corresponding differences are anticipated for the “bulk” of the layer and for the substrate-layer interface morphology between the two flight samples with respect to the results of ground-based tests. The ongoing characterization of the compositional and structural microhomogeneity of the flight and ground samples will further elucidate the effects of microgravity on the deposition and growth of epitaxial layers during the early state of growth. The information expected is of basic scientific and technological significance for vapor growth of epitaxial layers by this and other methods.

PRELIMINARY CONCLUSIONS: To date, the results of the USML-2 experiments are consistent with and extend the knowledge obtained from the USML-1 experiments and from ground-based studies, particularly with respect to the predicted critical transition time. The considerable morphological improvement of the surface of the epitaxial layer grown in microgravity compared to that obtained on the ground is highly significant, relative to similar improvements for much thicker layers grown during the USML-1 experiments. The results to date are very promising support of the investigators' predictions about the positive effects of microgravity on layer growth during the early stage of the deposition and growth processes. The above preliminary data suggest that the USML-2 experiments were successful. Further characterization of the two samples is in progress.

CRYSTAL GROWTH FURNACE

Interface Demarcation Flight Test (IDFT)

PI: M. Lichtensteiger

AFFILIATION: Universities Space Research Association, Marshall Space Flight Center, Alabama

PURPOSE: The purpose of the USML-2 CGF Interface Demarcation Flight Test task is two-fold. First, the integration of interface demarcation capability into the CGF system was tested under actual growth conditions, permitting the passage of well-defined current pulses of selectable repetition rate and duration, as well as switchable polarity through the growing sample with an amplitude of up to 40 Å.

This technique permits observation of the solid-liquid interface shape of the advancing growth form. The location of the interface, the shape of the interface, and, with a suitably encoded pulse sequence, the microscopic growth rate can be precisely determined. Figure 1 demonstrates the power of this technique. This information can be used to optimize growth conditions in the CGF in terms of furnace translation rates, choice of hot- and cold-zone temperatures, and the location of the sample in the furnace gradient.

Gallium-doped single-crystalline germanium was chosen as a model substance since its thermo-physical properties are well understood. Additionally, the extremely careful polishing and etching necessary to reveal this information on the surface of the lengthwise sectioned crystal have become an established procedure. Although non-trivial, since the amplitudes (heights) of the structures to be observed are in the nanometer range, differential interference contrast optical techniques allow a fairly ready determination of these features. An Atomic Force Microscope (AFM) scan of a small section of such a surface illustrates the point and is shown in Figure 2. The legends indicates a periodicity of approximately 12 μm, which correlates well with the measured growth rate, and further indicates an average height of approximately 10 Å for the current pulse-induced interface demarcation lines.

The effects of the additional energy input of the current pulses in the form of Joule heating on the location and shape of the original melt-back interface of the sample positioned in the thermally insulated furnace environment are also of practical interest. If the growth rate and interface shape information is correlated with the determination of dopant concentration on the micro-scale, the prevailing theories on crystal growth can be supported and/or augmented.

Second, the influences of a change from “CGF” to “GG” attitude during STS-73 on another steady-state growth system are to be assessed by using the demonstrated capabilities of the interface demarcation technique, which is briefly outlined above. Thus, it will be possible for the first time to quantify a tolerable deviation of the Shuttle microgravity environment from a true micro-gravity environment. This information should prove useful in defining future parameters for materials processing in space.

Interface Demarcation Flight Test (continued)

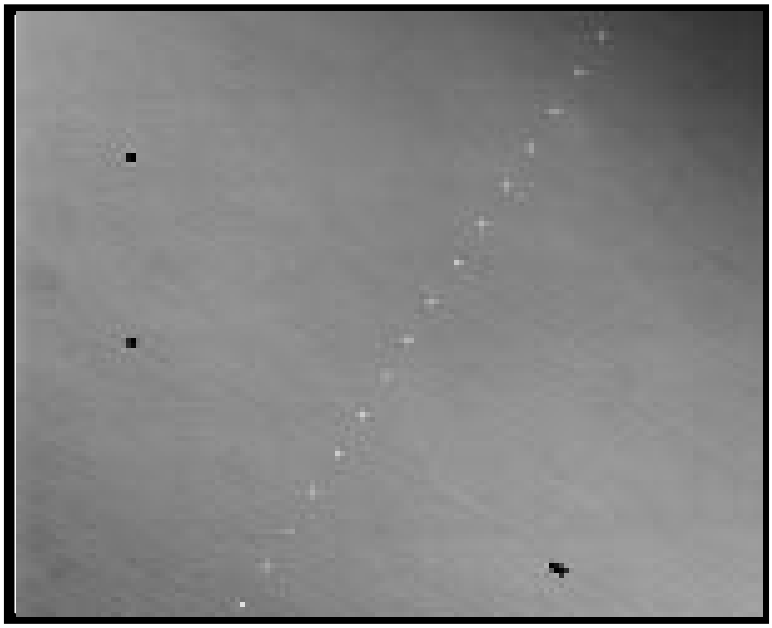
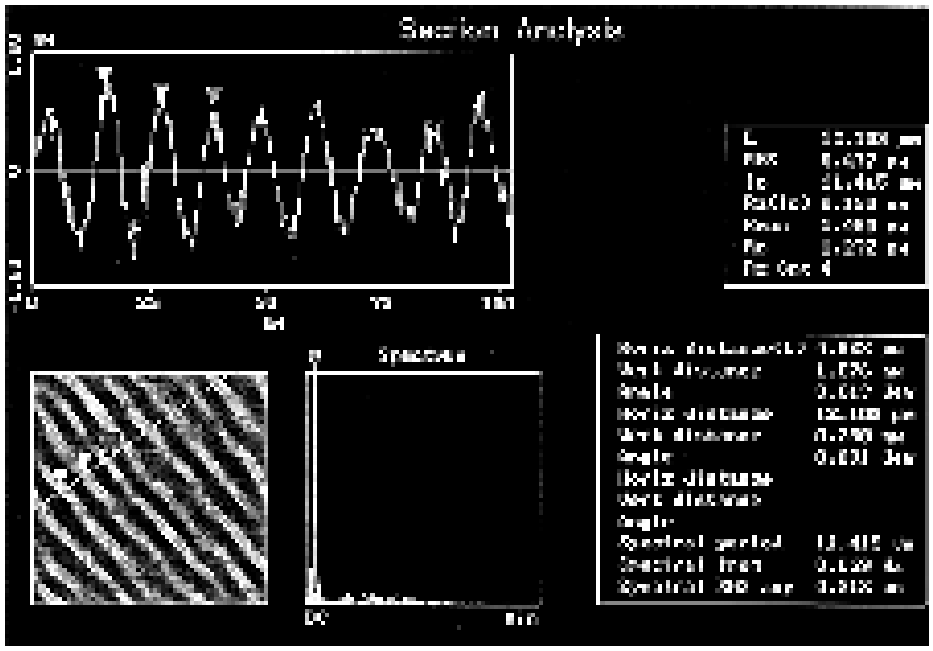


Figure 1. Micro-photograph of interface demarcation lines revealed by Nomarski differential interference contract. (Two double pulses, representing 60 sec of growth, are marked with dots. An inverted amplitude current pulse, marked with a short line, indicates an hour mark. The equally spaced bright dots are impact traces left by Spreading Resistance Profile measurements.)

Figure 2. AFM "screen dump" of steady-state growth section.



Area analyzed: 100μm x 100μm
Periodicity = Horizontal Distance = 12μm
Amplitude = Vertical Distance = 1nm

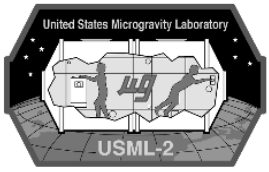
CRYSTAL GROWTH FURNACE

Interface Demarcation Flight Test (continued)

METHOD: Timeline adjustments permitted the growth of an additional sample with a translation-hold during the directional solidification process. Repeated thruster firings were required during growth to maintain “solar inertial” attitude during the 90-minute orbital periods. The hydrostatic pressure exerted on the solidifying melt during these high-g firings should lead to observable microscopic changes of surface morphology since the melt was forced into contact with the container wall; however, no clearly discernible features have been observed thus far, and detailed analysis will have to await the availability of sectioned and polished samples.

RESULTS: The original melt-back interface positions in both IDFT tests were within 0.5 mm of each other and were identical in the furnace-based coordinate system, which is well in accord with data obtained from a fluid-dynamics theoretical model of this experiment. The excellent thermal stability and performance of the CGF furnace developed jointly by Marshall Space Flight Center and Teledyne Brown Engineering are emphasized by this observation.

PRELIMINARY CONCLUSIONS: Currently, the ingots are being prepared for cutting into thin slabs parallel to the growth axis by a Burg+Mayer multiple-wire sectioning saw with minimal kerf loss. This process is to be followed by polishing individual plates to better than lambda/8 before initial analysis. This procedure will permit a three-dimensional reconstruction of the effects of attitude changes, thruster firings, and other flight disturbances experienced by the samples during growth.



DROP PHYSICS MODULE

Science and Technology of Surface-Controlled Phenomena

PI: R. Apfel

AFFILIATION: Yale University, New Haven, Connecticut

PURPOSE: This experiment had three major goals:

I. To determine the surface properties of liquids in the presence of surfactants (materials that migrate toward free surfaces or toward the interface between two liquids)

II. To investigate the dynamic behaviors and the coalescence of droplets coated with surfactant materials

III. To permit the study of the interactions between droplets and acoustic waves.

METHOD: For the first group of experiments, single liquid drops were introduced into the center node of the standing wave field in the near-ambient chamber of the Drop Physics Module (DPM) and were allowed to reach quiescent equilibrium. The gradual increase of the z-axis acoustic pressure caused the static deformation of drops. Shape oscillations about a spherical equilibrium shape were induced by a momentary release of the z-axis acoustic pressure. The amplitudes of the oscillations were determined by the initial aspect ratios of the drops. Shape oscillations about a controlled aspect ratio spheroidal equilibrium (axisymmetric) shape were induced by a single-step increase of the z-axis acoustic pressure. The variations of droplet shapes were recorded on videotape and cine film for later analysis.

In the second group of experiments, two liquid drops were positioned simultaneously at separate nodes of the standing wave field with a fork injector. They were brought together by appropriate adjustment of the two modes in the standing wave field. Coalescence will normally occur when two drops contact each other, but the surfactant can delay this process, as observed in the experiments. Three sample materials were investigated: triply distilled water, Triton-X-100 (a nonionic surfactant with fast surface absorption), and Bovine Serum Albumin (BSA, a nonionic surfactant with slow surface absorption).

RESULTS:

I. An automatic image analysis system was developed. The shapes of the oscillating drops recorded on videotape were analyzed frame by frame, revealing the variations of the oscillation amplitude versus time. The frequency and damping constant of the droplet shape oscillations were inferred using nonlinear fitting techniques. A total of 75 drop sequences with 28 drops have been evaluated. Well over 10,000 frames have been tallied thus far.

Science and Technology of Surface-Controlled Phenomena (continued)

II. The data from droplet oscillations about the spherical equilibrium shapes were applied to determine the surface properties (such as surface tension, surface elasticity, and viscosity) as functions of relative surfactant concentration.

III. The nonlinear (large-amplitude) shape oscillations of drops coated with surfactant materials were analyzed with the boundary integral method. A numerical program was developed to simulate the droplet shapes versus time.

IV. The data from the droplet oscillations about the deformed equilibrium shapes were used to develop a theoretical model of the shape oscillations of nonspherical drops.

V. The data of the droplet static aspect ratio versus the acoustic pressure were used in comparison with the theoretical calculations. Coalescence data have not yet been analyzed.

PRELIMINARY CONCLUSIONS: The capability to control droplet rotation in the DPM has greatly improved the quality of the data over that from USML-1. As hoped for, the majority of droplet shape oscillations in the experiments follow an exponential decay. The measurements show that the surface tension of the Triton-X-100 solution decreases obviously with the increase of concentration, but the surface elasticity and viscosity show only slight increases. For BSA solutions, although the surface tension has a small change with the variation of the concentration, the very fast damping of drop oscillations indicates that these solutions can have a large value of surface elasticity and viscosity. These results demonstrate that the surface properties of surfactant solutions depend strongly on both the surface sorption mechanisms and the molecular structures of surfactants.

The results of droplet shape oscillations with large amplitudes demonstrates that the oscillation frequencies shift toward a lower value when the amplitude is increased. The nonlinear effects, such as the energy exchanges between different modes, also become important. These are the first data of axisymmetric oscillations at such large amplitudes and, therefore, will be the standard for testing models for years to come.

The surface visco-elastic properties of surfactant solutions can modify the droplet hydrodynamic behavior, such as preventing fission of drops during extremely large amplitude super-oscillations. The experiment results of the shape oscillations of deformed drops display the decrease of the oscillation frequency and the increase of damping constant with the increase of droplet aspect ratio. These phenomena contributed to the interactions between the droplets and acoustic

Science and Technology of Surface-Controlled Phenomena (continued)

waves.

During the experiments, many of the drops still had slow rotation about the z-axis (less than 0.5 Hertz). Although the shift of the oscillation frequencies caused by this rotation is minor, its influence on the damping constant needs further investigation. The investigators will expand a theoretical model so that it can be used to estimate the changes of the damping constant versus the angular speed. Additionally, the current theoretical approach and numeric algorithm used in the prediction of droplet static shape in a one-dimensional acoustic field will be extended to the three-dimensional case; therefore, the droplet static shape in the DPM can be used to estimate the liquid surface tensions.

DROP PHYSICS MODULE

Drop Dynamics Experiment

PI: T. Wang

AFFILIATION: Vanderbilt University, Nashville, Tennessee

PURPOSE: The goals of this experiment were to gather high-quality data on the dynamics of liquid drops in low-gravity for comparison with theoretical predictions and to provide scientific and technical inputs for the development of new fields, such as containerless processing of materials and polymer encapsulation of living cells.

Drop dynamics research deals with the fundamental understanding of the behavior of liquid drops under the influence of external forces. These studies provide a basis for understanding scientific and technological areas in which liquid drops have a role, ranging from rain formation and weather patterns to chemical processing.

The experiments on the USML-2 mission included a study of the fission of rotating drops (the breaking of one drop into two drops) and a study of the centering mechanism in oscillating compound drops (how a drop of one liquid can be positioned at the center of a drop of a different liquid). Results from USML-2 should help develop theoretical models for the drop fission process. Information gathered on the centering mechanism of oscillating compound drops will help scientists understand the differences between theoretical predictions and data from the USML-1.

METHOD: Video and film records were made of the oscillating and rotating drops. These records are used to analyze the drop shapes, to obtain the oscillation frequencies, and to compare these data with theoretical predictions. The data are intended to allow determination of the equilibrium shapes and frequency spectrum of both simple and compound liquid drops undergoing different types of rotation and oscillation.

RESULTS: USML-2 was a very successful flight, and the investigators have approximately three times the amount of data to analyze as gathered on USML-1. The preliminary results for each experiment follow.

Drop Bifurcation and Fission

PURPOSE: On USML-1, the bifurcation of rotating liquid drops into two-lobed shapes was studied, and the data helped resolve long-standing differences between theoretical predictions and previous ground-based experiment results. These experiments laid the framework for experimentation on USML-2, emphasizing the need to understand clearly the role of acoustic flattening during bifurcation and the dynamics of the fission process as functions of drop parameters. Both these objectives were accomplished successfully on USML-2.

Drop Dynamics Experiment (continued)

RESULTS: Preliminary results from USML-2 show that acoustic flattening has strong influence on the bifurcation process. For small levels of flattening, bifurcation occurs at lower rotation rates, in accordance with the earlier results. As flattening increases, the bifurcation point shifts farther. Beyond a certain level of flattening, rotation affects the equilibrium of the drop, and the classical bifurcation route is not followed.

Centering of Compound Drops

PURPOSE: The goal of the USML-2 investigation was to study oscillation-induced centering of immiscible compound drops of large shell-core volume ratios (>4).

RESULTS: Preliminary results for such drops show that the coupling of the oscillations in the bubble mode is weak, and no centering could be effected in the bubble mode. In contrast, the compound drop could be driven to large amplitudes in the slosh mode, and centering could be effected.

Non-Linear and Chaotic Forced Drop Oscillation

PURPOSE: Data analysis of this experiment should provide new information on non-linear drop oscillations. Two fundamental goals of the study have been identified: hysteresis effect and jump phenomena and non-linear and chaotic drop oscillations. To achieve these goals, special measuring procedures -- frequency sweeps and multi-frequency excitation -- have been used.

RESULTS: Preliminary study of the videotapes recorded via the HI-PAC system showed clear evidence of transition from periodic to chaotic drop oscillations. Multi-frequency excitation has proven to be a suitable and efficient tool to bring the drop quickly into chaotic oscillation without excessive amplitudes while preserving levitation stability. Physically, the transition to chaos is enabled through ultra-subharmonic resonance and forced period doubling.

The preliminary observation of the frequency sweep measurements showed fast changes of the oscillation amplitude. The jump phenomena is a very abrupt change with a time scale of a few milliseconds.

Physical Acoustics and the Interaction with Dynamics of Drops

PURPOSE: The acoustic levitation of small liquid samples is often accompanied by instabilities such as uncontrolled rotation. To generate the greatest scientific return during USML-2, it was important to understand the origin of this phenomenon and its influence on the behavior of free drops.

RESULTS: Measurements were made by observing the displacement of a torsion pendulum introduced to the interior of the chamber. Spheres of various diameters were suspended from these fibers and their angular position measured optically using a charge-coupled-device (CCD) camera and image analysis software-based on a Macintosh computer. The

Drop Dynamics Experiment (continued)

correspondence between theory and measurement in the case of solid spheres supports the predictions of Busse and Wang.

Tumble Rotation Mechanism During USML-2

PURPOSE: This investigation was conducted as part of the Shape and Internal Flow Characterization Experiment. Upon deployment of a 4-cc water drop, a tumble along the x-axis was immediately initiated. The x-axis tumble predominated during USML-2 as opposed to the y-axis tumble reported during USML-1. The goal of this investigation was to provide a stationary drop with no linear or rotation translations. By comparison with pendulum measurements performed in ground-based experiments, this should have resulted in a counter torque of 0.05-0.10 dyne/cm applied to decelerate the motion of the drop.

RESULTS: During this experiment, the specimen may have been disturbed by other acoustic forces that were applied in an attempt to gain control of the specimen. The specimen slowed its rotation about the axis, undergoing an ill-defined motion as it approached zero velocity, and picked up a rotation in the opposite sense, again about the x-axis. This and subsequent observations will corroborate the description and understanding of the mechanism of the tumble rotation mechanism.

Polymer Membrane Formation in Space

PURPOSE: The objective of this experiment was to study the polymeric membrane (capsule) of the co-polymer poly HEMA-MMA in the convection-free environment. This pure diffusion experiment should reveal whether a membrane prepared under microgravity conditions exhibits a higher degree of homogeneity than one prepared under normal gravity conditions.

RESULTS: Characterization of the flight samples is proceeding, as well as simulations of flight experiments in terrestrial conditions.

Experiment #1: A droplet of 10 wt.-% poly HEMA-MMA solution in PEG was coalesced with the droplet of PBS solution inside the DPM. The polymer membrane started to form immediately after the droplets were brought together. The coalesced droplet was levitated for 30 minutes to allow the membrane to form in convection-free conditions. The experiment was repeated with a different volume ratio.

Experiment #2: This experiment was the same as Experiment #1 with two differences: the volume ratio (polymer solution: PBS) was equal to 1:1 when the droplet's diameter was ~2 cm, and the levitation time for this experiment was 15 minutes.

Drop Dynamics Experiment (continued)

Theoretical Studies

The Effects of Acoustic Flattening on the n=2 Rotational Bifurcation of a Drop: It was observed, in a plot of R* (dimensionless maximum radius) versus Ω* (dimensionless rotation rate) curve, that the main axisymmetric curve shifts upward with flattening because of acoustic radiation pressure. The axisymmetric rotational equilibrium curve and the shift in the n=2 bifurcation point will be calculated for a given level of flattening and compared with experiment results.

The Effects of Rotation on the Equilibrium of an Acoustically-Flattened Drop: It was observed that when a drop is drastically flattened by acoustic radiation pressure, it does not bifurcate by rotation but loses equilibrium at a critical rotation rate that depends on the given acoustic pressure. The shift in the maximum acoustic Bond number for equilibrium, as a function of the rotation Bond number, will be calculated and compared with experiment results.

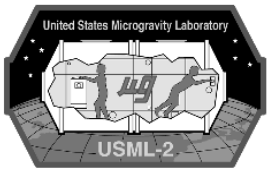
Dynamic Fission of a Drop after Losing Stable Equilibrium at the End of the n=2 Bifurcation Path due to Rotation: It was observed that the final fission of a rotating drop into two smaller drops involves stretching of the middle ligament to a maximum length (before snapping) that increases with viscosity. A one-dimensional viscous jet model will be used to study the dynamic process from the loss of equilibrium of the two-lobed shape to the moment of breakup, and the results will be compared with the experiments.

Centering of a Liquid-Core Compound Drop of Finite Volume Ratio in Large Amplitude Oscillations: It was observed that, for a liquid-core compound drop with a small core, it is easy to excite a sloshing mode oscillation but not a bubble mode oscillation. While centering of a liquid shell is essentially a Bernoulli effect, that of a compound drop is believed to be mostly a lubrication problem. Therefore, in the calculation for the centering of the liquid-core compound crop, the Bernoulli effect will be ignored with a concentration on the viscous effect. The results of the calculation will be compared to those of the experiments.

Multi-Frequency Excitations of Drop Oscillations Looking for Development of Chaos: It was observed that simultaneous excitations of the n=2 and n=4 modes in a drop can lead to chaos. The existing model will be refined by calculating the inertia matrix and evaluating the forcing function in terms of the imposed acoustic pressure. The predictions of the theory will be compared with experiment data.

Frequency Sweep in Excitation of a Drop Looking for Hysteresis and Jump Phenomena: It was observed that, during a frequency sweep, the resonance curve for the oscillations of a drop exhibits the expected jump effect.

GEOPHYSICAL FLUID FLOW CELL EXPERIMENT



PI: **J. Hart**

AFFILIATION: University of Colorado, Boulder, Colorado

PURPOSE: The goal of this experiment was to study how fluids move in microgravity as a means of understanding fluid flow in oceans, atmospheres, planets, and stars.

METHOD: The instrument was located in the Spacelab module and consisted of a stainless-steel hemisphere the size of a baseball, surrounded by a sapphire hemisphere. A layer of silicone oil was between the stainless steel and sapphire hemispheres. Sapphire was used because it is a unique material that conducts heat well and is transparent. This allowed precise temperature control and observations of the silicone oil as it flowed between the hemispheres.

An electric charge was applied to the fluid to create a buoyancy force identical to buoyant forces on Earth and in other atmospheres being modeled. The silicone oil contained a chemical that forms blue dye lines when subjected to ultraviolet light. Studying the movement of the lines allowed investigators to measure the fluid movement and velocity. Temperature measurements were made by studying the variations in the density of the fluid. Varying the rotation rate, temperature, and voltage in the facility created flows that are relevant to the study of oceans, planetary atmospheres, and stars.

RESULTS (INSTRUMENT PERFORMANCE): The Geophysical Fluid Flow Cell experiment carried out 29 separate 6-hour runs, using different parameters (cell rotation rate, heating distributions, and camera action types). Eighteen of the runs were nominal in terms of instrument performance, except for indications from the downlink electronics panel monitors that suggested higher than expected outer equator temperatures. This, and the following conclusions, are based on review of the time-lapse video recording and other information obtained real-time at the Payload Operations Control Center. Because of the over-temperature problem, science activities were refocused on situations with spherically symmetric heating (the so-called “solar model” cases) and those with only weak latitudinal gradients. Several of the runs with low rotation rates contained many surprises, in particular observations of unanticipated states of motion, so this off-nominal performance of the GFFC thermal controller was not necessarily detrimental to GFFC science. The last 11 runs were affected by the apparent loss of the 16-mm film single-frame film transport; thus, it is likely that these runs have only video data, which do not contain the detailed instrument parameters, e.g., most importantly, temperature measurements at various latitudes, normally recorded only on the 16-mm film. This loss was compensated for by repeating experiments run earlier in the mission, for which the film transport worked properly, and by increasing downlink monitors of the corner temperatures. Using this approach, the last 11 runs still contributed significantly to the overall science goals of the GFFC experiment.

GEOPHYSICAL FLUID FLOW CELL EXPERIMENT

RESULTS (SCIENCE): The following are the preliminary results based on video downlink recorded during USML-2. Film data have yet to be received. The experiments fell into several classes depending on the rotation rate (rapid or slow: e.g., solar-like or mantle-like) and the heating distribution (spherically symmetric or varying in latitude: e.g., solar/Earth core versus Jupiter's or Earth's atmosphere). In each case, new states were observed and are summarized below.

I. Studies of rotating convection with spherically symmetric heating revealed possible multiple jets in latitude, with prograde (same sense as the basic rotation) motion of thermal waves at low and high latitudes and retrograde pattern rotation at mid-latitude (Figure 1). Such differential pattern propagation has not been seen previously in computational models, and these results may provide an alternative view on the mechanisms for "banded"-looking structures in planetary atmospheres like that of Jupiter.

II. A search for "climatic" states in slowly rotating convection was carried out, and two distinct convection patterns were observed in experiments with the same external parameters but with different initial conditions. This means that the long-time evolution of modestly convecting flows in slowly rotating spherical shells (like Earth's mantle) are not unique but depend on initial conditions. Equivalently, the "climate" (or long-term thermal patterns) can be persistent, or locked, for a long time as external conditions are varied. In addition, unique information was obtained on how these persistent states break down as parameters are changed. Figures 2a and 2b illustrate the instability of "horseshoe convection," wherein the off-center ring of convection breaks down by north-south stripe formation as the voltage is increased from 1.44 kV to 1.56 kV.

III. A large data set (several rotation rates, many different heating rates) was obtained on the transition between anisotropic north-south "banana convection" and more isotropic non-aligned convection. These results, when quantified by digital analysis of the data films and tapes, will permit testing of simple scaling arguments for this transition. Once verified, these scalings can be used to classify the expected global convection regimes of planets and stars.

IV. Experiments with latitudinal heating gradients showed evidence for baroclinic waves. This instability is interesting because it has combined attributes of both ordinary thermal convection and rotating slantwise convection, such as occurs in Earth's atmosphere.

V. Other experiments with latitudinal heating successfully showed how spiral wave convection breaks down to turbulence by secondary branching instability.

GEOPHYSICAL FLUID FLOW CELL EXPERIMENT

PRELIMINARY CONCLUSIONS: The GFFC experiment met most of its objectives. The planned experiments with high-latitudinal temperature gradients, which were not run because of thermal regulation problems, were replaced with low-rotation “mantle convection climate-state” investigations that produced interesting and surprising results with respect to persistent locked regimes and their instabilities. Future data processing efforts, when the data films and on-board video tapes become available, along with computer simulations, will shed light on a number of scientifically important issues.

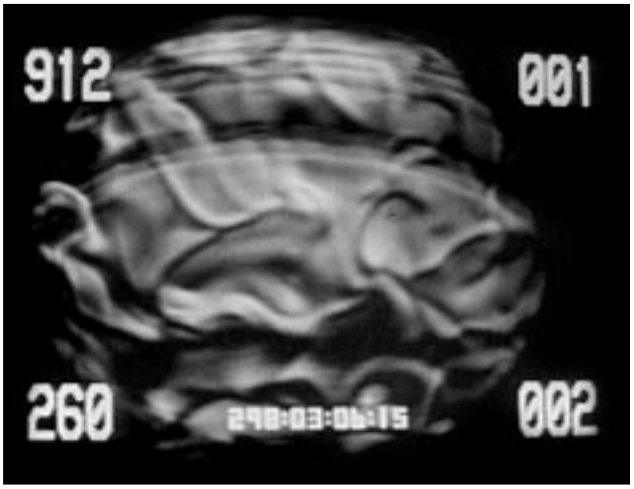


Figure 1: Above is a turbulent solar model case. The equator is at the top, and the pole is at the bottom. The features propagate prograde at the bottom and top of the frame, while moving retrograde at mid-latitude.

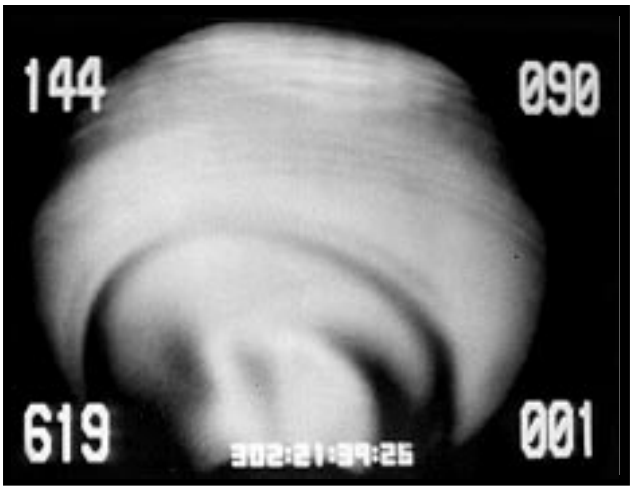


Figure 2: This stable low-rotation convection state consists of an off-polar convicting ring.

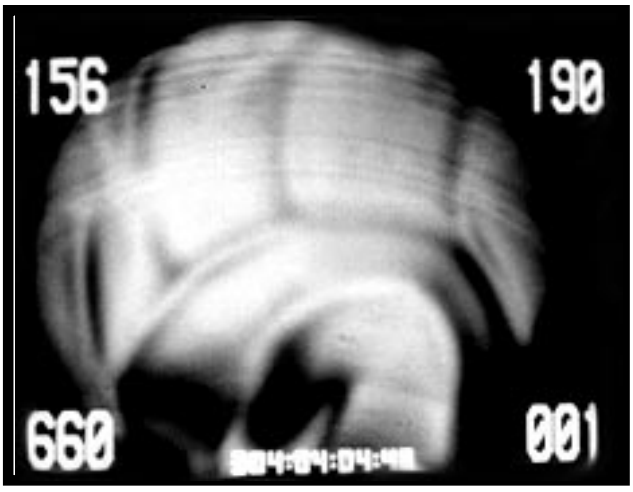


Figure 3: As the voltage is increased, this nearly axisymmetric state breaks down as columnar modes appear.

GLOVEBOX

The Spacelab Glovebox, provided by the European Space Agency, offered investigators the capability to carry out experiments, test science procedures, and develop new technologies in microgravity. It enabled crewmembers to handle, transfer, and otherwise manipulate experiment hardware and materials in ways that are impractical in the open Spacelab. In addition, the facility was equipped with photographic equipment that allowed a visual record of experiment operations. Many investigations benefited from the increased crew involvement and the photographic and video capabilities of the facility.

The Glovebox has an enclosed cabinet that offers a clean working space and minimizes the risk of contamination to the Spacelab, the crew, and the samples. It provides physical isolation and a negative air pressure differential between the enclosure and the rest of Spacelab. An air-filtering system also protects the Spacelab environment from experiment products that could be harmful to the crew.

The Glovebox provides the following features to microgravity experiments: a large viewing window atop the cabinet, experiment mounting and positioning equipment, real-time downlink of experiment video and housekeeping data, electrical power, partial temperature control, time-temperature display, lighting, and cleaning supplies. Two color and two black-and-white channels are available to record experiment operations and the behavior of specimens.

The crewmembers manipulated samples and equipment through three doors: two glovedoors and one central port through which experiments were placed in the Glovebox. The glovedoors are located on each side of the central port.

There were seven separate investigations carried out in the Glovebox facility. Details on each are included on the following pages.

GLOVEBOX INVESTIGATIONS

Colloidal Disorder-Order Transitions (CDOT)

PI: P. Chaikin

AFFILIATION: Princeton University, Princeton, New Jersey

PURPOSE: The CDOT experiment on USML-2 attempted to measure the fundamental properties of a colloid at the crystallization phase boundary where it is solidifying from a liquid in microgravity. Colloids are suspensions of finely divided solids in gaseous or liquid fields. For example, paints, inks and ceramics are colloids in the fluid-like phase before they dry or set. Researchers want to know what is occurring at the boundary between the liquid and solid states of a colloid. This will help in understanding the basic interactions driving the crystalline transition and in determining the structure and properties of all solids in all materials. It also will improve materials processing on Earth, as well as in microgravity.

METHOD: Fifteen colloidal samples of ~0.5 micron spheres of PMMA suspended in a mixture of decalin and tetralin (to match the optical index of refraction) were homogenized after launch, setting them into an unstable liquid phase. Then, they were allowed to re-form in microgravity. Three and five days after homogenization, digital and 35-mm photos were obtained to assess the degree of crystallinity and the size and shape of the crystals. Seven days after homogenization, a complete set of CDOT experiments was performed, including measurements of the static and dynamic light scattering and the shear modulus. The static scattering, which is used to determine the detailed crystal structure, was measured using a laser beam radially incident on the cylindrical sample cell. The laser light is Bragg scattered from the sample onto a semi-circular cylindrical screen and is observed with a CCD camera and recorded. The laser light also is detected by a photodiode and is processed with a correlator to obtain information on particle motion. The sample is oscillated and stopped. The decay of the oscillation, as detected on the scattering screen, yields information about the elastic properties (shear modulus) of the sample. The sample is translated to illuminate different regions and to obtain averages for the measured quantities.

RESULTS: Data were successfully obtained for all of the flight experiment samples. Digital and 35-mm photography of 15 samples was obtained. While investigators have not received the 35-mm photos yet, the digital images downlinked during the flight show most of the expected behavior but with interesting surprises. The crystalline samples look similar to ground-based results but have larger crystallites. The samples grown in the liquid-solid coexistence region, on the other hand, show large solid "butterfly-like" structures indicating dendritic growth of crystallites suspended in the fluid phase after 2 and 3 days of growth. This growth

Colloidal Disorder-Order Transitions (continued)

mechanism has not been observed on ground-based samples. (It will be most interesting to observe the 35-mm pictures that were taken after 12 days of growth.) Another surprise was the crystallization in space of a sample that remained in the glass state under normal gravity.

The static laser light scattering experiment showed very bright, strong Bragg spots and Bragg streaks for the crystalline samples. The intensity of the scattering and the narrowness of the structures observed on the scattering screen confirm that the crystallites grew larger in microgravity; however, the streaks that were observed on the Shuttle experiment were much more dominant and pronounced compared to ground-based experiments on the identical samples. This leads investigators to believe that the crystallites grown in 1-g are a mixture of Face Centered Cubic (FCC) and Random Hexagonal Close Packed (RHCP) structures, while in microgravity the mixture is more dominated by RHCP than by FCC.

The "pinging" of the sample cells produced oscillations in the Bragg scattering image, indicating the existence of a finite shear modulus in the crystalline samples. The dynamic light scattering appeared to perform well for all the samples, including liquid and glass states. Preliminary analysis on shear modulus and dynamics indicate roughly similar dependencies with ground-based results. The differences must await analysis of the complete set of data, which investigators have not obtained to date.

Possibly the most interesting samples, those which exhibited dendritic growth in the coexistence region did not survive return to gravity (landing), as expected; however, the crystalline and glass samples did survive landing, and investigators are monitoring their evolution in time. Preliminary results indicate that the RHCP structure is giving way to smaller crystallites with a FCC structure.

PRELIMINARY CONCLUSIONS: Although only part of the data collected during the Shuttle experiment is currently available, some tentative conclusions can be drawn. The tendency toward RHCP in the microgravity experiments, at least at early times after nucleation, probably is coupled with the non-equilibrium dendritic growth that was observed for the crystals in the coexistence region. In all probability, dendritic or nonequilibrium growth dominates in all regimes and leads to a random, nonequilibrium structure RHCP. When the crystallites are allowed to anneal (on Earth, unfortunately), they tend to re-form as FCC. In doing so, they nucleate small FCC regions, producing broad Bragg scattering peaks, which destroy the narrow RHCP streaks formed from the initial non-equilibrium growth.

Colloidal Disorder-Order Transitions (continued)

Investigators speculate that the existence of the glass state, as a separate thermodynamic state, is much more tenuous than previously thought. Although samples that are deep into the glassy region of the phase diagram have been used, the microgravity experiments have shown that, in the period of a week, one can attain the crystalline state. Results suggest that the glassy state is distinguished from the crystalline state simply by the amount of time that it takes to nucleate a critical droplet. This effect is somewhat masked in the presence of gravity since sedimentation increases the concentration and decreases the rate of nucleation faster perhaps than the time required for the nucleus to form at any given density.

One of the main conclusions is that the coexistence region and the glassy region need study for a very extended period of time (months to years) in a microgravity environment to clarify the fundamental physics of this system.

Interface Configuration Experiment (ICE)

PI: **P. Concus**

AFFILIATION: University of California, Berkeley, California

PURPOSE: The purpose of this experiment was to explore the behavior of equilibrium liquid-vapor interfaces in low gravity.

METHOD: The phenomenon of interest for this experiment concerns the discontinuous or nearly discontinuous behavior of an equilibrium-free surface of liquid partly filling a container in which large shifts of the liquid mass can result from small changes in container geometry or contact angle. The two types of cylindrical containers used for the study were a movable wedge and three mathematically derived cross-section (proboscis) containers. The containers were partially filled with liquid, and the shapes assumed by the liquid were observed and recorded initially and after disturbances were applied and/or the wedge angle was varied.

RESULTS: Downlinked video segments during the experiment indicate that the displacement of liquid from the reservoirs to the containers proceeded satisfactorily for all vessels. Initial sticking of the piston/O-ring assembly resulted in some small blobs of liquid being ejected onto the container walls of the movable wedge vessel, but the remainder of the injection went normally and smoothly. For the three proboscis vessels, all injections went smoothly from the beginning.

For the movable wedge vessel, qualitatively different behavior could be observed for values of the interior wedge angle on opposite sides of a critical value, as predicted by the idealized mathematical theory. When the wedge angle was decreased below this critical value, the fluid advanced rapidly along the vertex toward the top of the container. By suitably opening the wedge angle before the fluid had reached the top, the rise could be halted. A difference in the advancing and receding profiles indicated the effects of hysteresis on the phenomena. Analysis of the video recordings is expected to yield more detailed, quantitative information.

For the proboscis vessels, careful tapping of the containers by a crewmember showed that differing behavior could be observed on the two sides of the critical contact angle. In the second vessel, the critical angles for the two proboscides differed by only 4 degrees, and the measured equilibrium contact angle of the fluid was about halfway between the two. In this vessel, there was clear evidence that, in the smaller, critical angle proboscis, the liquid could not be coaxed to rise up the vertex by moderate sloshing of the liquid but that for the larger critical angle it could. As above, the video recordings for the three vessels are being analyzed to describe this interesting behavior more closely.

PRELIMINARY CONCLUSIONS: The discontinuous behavior phenomenon mathematically derived from the idealized classical model could be observed qualitatively for a real fluid, even in the presence of surface friction (hysteresis) effects. Study of the videotapes is expected to provide detailed information on these effects.

GLOVEBOX INVESTIGATIONS

Protein Crystal Growth - Glovebox (PCGG)

PI: L.J. DeLucas

AFFILIATION: The University of Alabama at Birmingham, Alabama

PURPOSE: The general purpose of the Glovebox Protein Crystal Growth experiments is the same as that of the Commercial Protein Crystal Growth (CPCG) project: to produce large, well-ordered crystals of various proteins under controlled conditions in microgravity. The Glovebox PCG experiments also provided the opportunity to optimize protein crystal growth experiments on orbit and provided real-time video and electronic still images of crystal growth experiments to scientists on the ground.

METHOD: The vapor diffusion method was used to grow crystals of seven proteins in individual growth chambers. The hardware provided the capacity for 150 separate experiments. The experiments were set up in the Glovebox and were then stored at 22 ˚C in a Commercial Refrigerator/Incubator Module (CRIM) in the Shuttle's middeck. Based on initial screening experiments, the crystal growth conditions were optimized and additional experiments were set up by crewmembers in the Glovebox. This iterative process was continued until flight day 7, and then the crystals were allowed to grow until deactivation of the experiments on flight day 13.

RESULTS: Crewmembers Dr. Cady Coleman and Dr. Al Sacco set up more than 80 individual experiments in the Glovebox, and many of those experiments produced diffraction-sized crystals. The following list summarizes the results of these experiments.

PROTEIN RESULTS

Microsomal Triglyceride Transfer Protein:
Several well-formed cubic crystals up to 0.3 mm on each edge

IMP Dehydrogenase:
Small, thin rod-shaped crystals up to 0.2 mm long

Feline Calicivirus:
Many perfect rectangular and square pyramidal crystals up to 0.6 mm in length

Lysozyme:
Well-formed tetragonal crystals up to 0.7 mm in length

Collagen Binding Domain:
Clusters of long, needle-shaped crystals too thin for X-ray diffraction studies

Staphylokinase:
Fine, crystalline precipitate, but no usable crystals

Duck Delta II Crystalline:
Square pyramidal crystals up to 0.5 mm long; several triangular wedge-shaped crystals that possibly could be a new morphology

Protein Crystal Growth - Glovebox (continued)

The feline calicivirus crystals have been stored while the Principal Investigator, Dr. Ming Luo, awaits time at a synchrotron facility to collect X-ray data. Dr. Lynne Howell, Principal Investigator for the duck delta II crystalline project, reported that the space-grown crystals of this protein did not diffract. This phenomenon has not been experienced previously with ground-grown crystals and is thought to be a negative impact of the extended launch delays. Laboratory experiments are being conducted to reproduce the results obtained in the Shuttle experiments. X-ray diffraction data have been collected on the crystals of microsomal triglyceride transfer protein, IMP dehydrogenase, and lysozyme, but analyses of these data are not complete.

The crewmembers experienced problems with bubbles in the precipitant and buffer solutions but worked creatively to minimize the effect of the bubbles on the experiments. Although they worked quickly and efficiently, time was the major determinant in the number of experiments that were completed.

Early problems with the interior Glovebox temperature were resolved by lowering the avionics air temperature, and the Glovebox PCG hardware worked very well. During the flight, crewmembers downlinked video of individual experiments and interacted with the CPCG science team to design new experiments to optimize crystal growth. Observations and calculations made by the crewmembers were stored in Excel files and were downlinked by the Ku-band Communication Adaptor (KCA). This interaction established a valuable scientific link between the payload crew and the science team and greatly enhanced the success of these experiments.

PRELIMINARY CONCLUSIONS: Preliminary analysis indicates that the Glovebox PCG experiments aboard USML-2 were quite successful, producing diffraction-sized crystals in five of the seven proteins flown. The Glovebox Protein Crystal Growth hardware worked very well and provided the opportunity for on-orbit optimization of crystal growth conditions. The video downlink, electronic still camera image transfer, and the KCA file transfer provided invaluable interaction between the Shuttle crew and the science team.

GLOVEBOX INVESTIGATIONS

Oscillatory Thermocapillary Flow Experiment (OTFE)

PI: Y. Kamotani

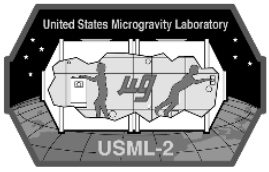
AFFILIATION: Case Western Reserve University, Cleveland, Ohio

PURPOSE: This experiment investigated the conditions for the onset of oscillations in thermocapillary flows. It studied the effect of test chamber depth on the onset conditions to complement the Surface Tension Driven Convection Experiment (STDCE).

METHOD: Thermocapillary flows were generated in cylindrical test chambers filled with 2 centistokes silicone oil. Four test modules were used to study two different diameters (1.2 and 2 cm) and two different chamber aspect ratios (ratio of chamber depth to radius = 0.5 and 2). A submerged heating rod was used to heat the fluid and to generate oscillatory thermocapillary flows. Aluminum oxide particles were mixed in the fluid, and the particle motion was observed through a video camera to identify the onset of oscillations.

RESULTS: The OTFE completed all four tests successfully. Oscillations were found in all the tests. The critical temperature difference for the onset of oscillations increased as the chamber aspect ratio was decreased. The conclusion was drawn by comparing the OTFE and STDCE data. The aspect ratio effect was consistent with the trend found in the STDCE for the laser heating mode.

PRELIMINARY CONCLUSIONS: The video tapes are being analyzed to obtain quantitative results. Investigators will analyze the effect of aspect ratio on the onset conditions, as well as on the oscillation frequency. All the results from the OTFE and STDCE will be combined, analyzed, and interpreted to explain the oscillation phenomenon.



GLOVEBOX INVESTIGATIONS

Particle Dispersion Experiment (PDE)

PI: J. Marshall

AFFILIATION: NASA Ames Research Center, Moffett Field, California

PURPOSE: The experiment investigated the electrostatically driven aggregation of fine granular materials to determine aggregation rates, aggregation modes, and the geometry of electrical fields generated by the aggregates themselves. Particle-cloud density, dielectric properties of the grains, and grain shape and size were variables in the tests. The experiments should shed light on the role that aggregation plays in affecting the collapse of dust and debris clouds in astrophysical and planetary settings. Particulate clouds occur in protostellar and interstellar nebulae, in planetary rings, and in planetary atmospheres as ephemeral products of meteorite impact, volcanism, firestorms, and aeolian activity. They also are produced by anthropogenic agencies of nuclear detonation and industrial emissions.

METHOD: Clouds of grains were generated inside self-contained modular experiment chambers in the Glovebox facility. The clouds were produced in 8 separate chambers (of 125 cubic centimeters each), using an air pulse to distribute grains around the experiment volume evenly. The dispersion also was aided by the manipulation of the modules by the crew. Aggregation of the grains into various types of clumps occurred naturally from these clouds and was recorded on video throughout the duration of the tests. High-magnification video images also were acquired of the aggregates, using the Glovebox microscope.

RESULTS: Aggregation of grains was observed to occur virtually instantaneously from the dispersed clouds. Particulate clouds of quartz and volcanic ash produced aggregates in the form of chains or filaments that were only one grain in width but many tens of grains in length. In the best case observed, 400-micron grains formed single chains of greater than 1 cm in length. Bifurcating and fractal three-dimensional variations of the filaments were observed. Copper grains used as experimental controls also produced filamental aggregates. This was an important and surprising result because metallic (electrically conducting) particles were expected to behave differently. Cloud density (the number of grains per unit volume) affected aggregation by giving rise to more abundant and longer aggregates in denser clouds. Grain size and grain shape were not observed to influence the aggregation process significantly. Both the way in which the grains came together in the aggregates and the geometry of the aggregates themselves strongly implicate electrical dipoles as the cause of aggregation into filamental structures. This also may be true for the metallic grains that could have been held together by dipoles generated from residual charge differentials at grain-grain contacts.

Particle Dispersion Experiment (continued)

PRELIMINARY CONCLUSIONS: The instantaneous nature of aggregation, the efficiency with which it consumes cloud particles, the universality of aggregation for both conducting and non-conducting materials, and the growth of electrical fields around the aggregates are all factors suggesting that aggregation should be a potent influence on the behavior of dust and debris clouds. Aggregation should reduce cloud lifetimes and, in some cases, may lead to catastrophic collapse of the cloud system. Particle clouds associated with volcanic eruption plumes or with dust storms on Mars and even those of protostellar nebulae are known to undergo relatively rapid and unpredictable collapse; aggregation may be involved.

Zeolite Crystal Growth - Glovebox (ZCGG)

PI: A. Sacco

AFFILIATION: Worcester Polytechnic Institute, Worcester, Massachusetts

PURPOSE: The Zeolite Crystal Growth (ZCG) experiments performed in the Glovebox were companion investigations to the middeck ZCG experiments. The Glovebox ZCG investigations helped maximize the science return of the primary ZCG experiment, leading to new strategies and methodologies for improving Zeolite growth in orbit. The Glovebox ZCG experiments used clear autoclaves to determine the proper number of times the fluids should be worked to ensure proper mixing.

(See Zeolite Crystal Growth experiment write-up for a complete description of the combined experiment.)

GLOVEBOX INVESTIGATIONS

Fiber Supported Droplet Combustion (FSDC)

PI: **F.A. Williams**

AFFILIATION: University of California, San Diego, California

PURPOSE: Droplet combustion studies are very difficult to perform on Earth. Gravity causes high-density droplets to sink, and buoyancy-induced acceleration forces combustion products to rise, resulting in drops that burn unevenly. Employing a new technique for conducting droplet combustion studies, this experiment uses a very fine fiber to keep the droplet positioned in the field of view of the measurement instrumentation, giving researchers access to a tool for studying fundamental combustion processes, such as how pollutants are formed. The purpose of the experiment is to test the new experiment techniques and to obtain new data on droplet combustion that can improve current understanding of the associated combustion processes.

METHOD: The apparatus consists of a test flight unit 20 cm high, 17.5 cm wide on the front and top faces, and 15.5 cm wide on the end faces. On one end face is a fine mesh screen, and on the other end face is a fan to draw air through the experiment. In front of the fan is a sheet of fine porous material to provide the necessary pressure drop to get the required low-velocity flows within the test chamber. A droplet is grown on the fine silicon carbide fiber by opposed fuel needles fed by the fuel accumulator system. The opposed needles are retracted simultaneously, leaving the droplet on the fiber. Subsequently, a hot wire ignites the droplet, and then the wire is retracted back to the floor. The droplet combustion is recorded by video, in a top view with backlight through a microscope to obtain droplet diameters and also in a front view without magnification to obtain flame diameters, both as functions of time during combustion.

RESULTS: A series of tests with methanol droplets was conducted. Successful tests included 2-, 3-, 4-, 5-, and probably a 6-mm droplet burning in quiescent air. The larger droplets burned for more than 30 seconds. In fact, the extinction diameter of the largest droplet was larger than the initial diameter of droplets burned on the ground. These data will allow the first comparison of theoretical predictions of extinction diameters for droplets of this size. In a few cases, a low-speed air flow was started after the droplet was ignited. This sharply increased the flame luminosity and changed the flame shape from spherical to ellipsoidal, as expected. These data will serve as the first data against which an axisymmetric numerical model (in development) will be compared. Finally, a few tests were run with different fiber diameters (80 and 140 μm), from which the investigators will ascertain if the fiber size made a difference in these droplet-burning tests.

Fiber Supported Droplet Combustion (continued)

In addition to the methanol tests, several series of tests with fuel mixtures were conducted. In the first test, initial water content in the methanol fuel droplets was 10% and 20% by mass. The tests showed, in comparison with pure methanol, that the burning rate diminished, and the extinction diameter increased in these tests in qualitative agreement with predictions. Next, a series of tests with sooting fuel mixtures was performed. The first sooting fuel mixture was heptane/hexadecane. A droplet approximately 5 mm in diameter issued enormous amounts of soot, far more than expected. This soot passed through the flame surface without being fully oxidized. Most the subsequent testing was done with smaller droplets to lessen the amount of soot produced. These exhibited very large aggregates of soot surrounding the droplet. These tests showed that the initial diameter seemingly has a large effect on the amount of soot produced. Similar findings were obtained in the final test runs with methanol/dodecanol fuel mixtures.

PRELIMINARY CONCLUSIONS: Preliminary principal conclusions are that the methanol extinction diameters are in approximate agreement with theoretical predictions, while the sooting of larger heptane-hexadecane droplets is much heavier than was initially anticipated from theory. These results are being analyzed much more thoroughly now to improve accuracies of burning rates and extinction diameters and to determine the cause of the heavy sooting.

PROTEIN CRYSTAL GROWTH

Single-Locker Protein Crystal Growth (SPCG)

PI: D. Carter

AFFILIATION: NASA, Marshall Space Flight Center, Alabama

PURPOSE: The purpose of these experiments was to grow small quantities of various proteins in a single-locker thermal enclosure system using the vapor diffusion method.

RESULTS: A wealth of diffraction-size or better crystals were produced for all proteins flown in the Diffusion-Controlled Protein Crystal Growth Apparatus for Microgravity (DCAM) and the Protein Crystallization Apparatus for Microgravity (PCAM). Approximately 50% of these samples produced crystals in microgravity that exhibited at least one significant improvement over their Earth-grown counterparts.

Three of the proteins produced crystals that are much larger in size when compared to other crystals previously grown by this or any other method. In one case, the Earth-grown crystals were not large enough to study. Current status and details of the individual experiments are given below.

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HIV Protease Complexed with Inhibitor

Purpose: To improve the quality of protein crystals for increased resolution to aid in the design of an inhibitor against HIV

Results: A complete diffraction data set has been collected from a selected crystal. Diffraction data exhibit superior R-factors in shells of resolution, overall enhanced I/sig.I ratios, and improved resolution. Refinement of the drug complex is in progress to evaluate any improvements in the resulting electron density.

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Raf Kinase

Purpose: To determine the three-dimensional structure of the N-terminal regulatory domain of Raf Kinase. Raf Kinase is a Ser/Thr Kinase involved in the signal transduction cascade that originates with a receptor at the surface because of binding with a growth hormone.

Results: Status is not available at the time of inquiry.

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c-Phospholipase A2

Purpose: To improve the diffraction quality of the crystals to provide structural information to be used in structure-based drug design

Single-Locker Protein Crystal Growth (continued)

Results: Crystals of c-Phospholipase were the largest ever produced, approximately an order of magnitude larger in a single dimension. Previously, crystals were not large enough to pursue the structure. Analysis is in progress. This is potentially an extremely important example of "enabling" microgravity research.

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L-Alanine Dehydrogenase from *Bacillus subtilis*

Purpose: To improve the diffraction quality of the crystals to aid in structural determination

L-alanine dehydrogenase catalyses the reversible oxidative deamination of L-alanine to pyruvate and ammonium. This enzyme does not seem to share sequence similarity with other amino acid dehydrogenases whose three-dimensional structures are known but seems to be structurally related to the transmembrane proton translocating pump, pyridine nucleotide transhydrogenase. The three-dimensional structure will allow a better understanding of the catalytic reaction and of the enzymologic and structural relationships between these proteins.

Results: The largest crystals of L-alanine dehydrogenase ever grown were produced, along with a new morphology and space group. Tests on the synchrotron elucidated the space group but did not provide the desired resolution. However, the Co-Investigator remains extremely interested in pursuing this further, because the Earth-grown crystals are too small for study.

Human Cytomegalovirus Assemblin

Purpose: To increase crystal size and quality for greater resolution data to aid in drug design

Human Cytomegalovirus (HCMV), in addition to other members of the herpes virus family, encodes a unique serine protease, assemblin, that is necessary for viral replication. Accordingly, specific inhibitors of HCMV assemblin should interfere with the replication of this increasingly important human pathogen. Crystallization and structure determination of assemblin will provide information necessary for the design of anti-viral drugs. The assemblin target forms the basis for a high-priority, structure-based drug design project at Monsanto/Searle.

Results: Crystals of two variants of assemblin were grown, and numerous diffraction-sized crystals were produced. Data thus far indicate improvement in mosaic spread (internal order) but not in resolution. Extended delays in the post-flight analysis were experienced because of X-ray equipment failure at Monsanto. Samples may have deteriorated by the time analysis was performed.

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Human Antithrombin III

Purpose: To improve crystal quality, size, and morphology to obtain higher resolution data to aid in the design of antithrombin therapeutics

Single-Locker Protein Crystal Growth (continued)

Antithrombin is a member of the serpin family of serine protease inhibitors, which are larger than the other inhibitor families and are characterized by the remarkable flexibility of their relatively long reactive center loops. Antithrombin has 432 amino acids organized into 9 helices and 3 b-sheets with 3 disulfide bonds and 4 N-linked oligosaccharide chains. Its physiological function is to control blood coagulation in human plasma, which it does by forming inhibitory complexes with thrombin and other coagulation proteases in a process greatly accelerated by heparin. Its importance is underscored by the occurrence of severe thrombotic disorders, including deep vein thrombosis, pulmonary embolism, and cerebral infarction in subjects with antithrombin mutations that result in either decreased plasma levels or aberrant inhibitory function. Antithrombin also is commonly given as supplementation therapy to patients suffering the thrombotic crises of shock syndromes.

Results: Crystals grown on an earlier Shuttle flight produced crystals in the PCAM superior to any previously grown by any other method. This has resulted in the ability to fit and refine the atomic model, which had not been possible. Additionally, crystals of this protein complexed with effectors were sought from this mission to study the molecule's chemistry. Because of a series of long delays, this fragile protein did not crystallize well after the third reload; however, crystals were produced, and analysis is in progress. This is a beautiful example of "enabling" microgravity research on an extremely important, medically significant protein.

Gro EL Protein/Bacteriophage HK97 Capsid Protein Complex

Purpose: To improve the diffraction quality of the crystals to aid in structural determination

Results: Large crystals were grown, with no improvement in diffraction reported.

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Lysozyme

Purpose: To improve crystal quality for greater resolution of the atomic model for documentation of the effects of microgravity on crystal growth and to characterize microgravity performance of DCAM

Results: Numerous high-quality lysozyme crystals were produced in the PCAM and DCAM assemblies. Several data sets were collected, further documenting improved order and improved resolution; additional analyses were performed. A manuscript describing the refined high resolution has been completed. This work, combined with two previous flight PCAM flight samples, has produced the highest resolution (atomic structure) refinements of hen egg white lysozyme to date. Important improvements were gained in R-factor, water structure, and thermal parameters. A few extremely large, well-formed crystals were produced by selected growth profiles in the DCAM apparatus.

Single-Locker Protein Crystal Growth (continued)

Nucleosome Core Particle

Purpose: To grow larger, better quality crystals to aid in structure determination

The nucleosome is the fundamental structural unit of chromatin and is the basis for organization within the genome by compaction of DNA within the nucleus of the cell and by making selected regions of chromosomes available for transcription and replication. Nucleosomes represent a protein-DNA complex found in all eukaryotes that has been considered to be non-specific with regard to the DNA sequence and nonregulatory in function. Scientists now know that certain DNAs, such as the human alpha-satellite DNA used in this experiment, contain subtle sequence characteristics that allow the core histones to bind in a specific phase on the sequence. They also know that nucleosomes have important roles in the regulation of gene expression, particularly in the expression of genes transcribed by RNA polymerase III.

The nucleosome core particle has been defined on the basis of protection of DNA from nuclease attack. The nucleosome core particle consists of two units each of the core histones, H4, H3, H2A, and H2B, along with approximately 146 base pairs of double helical DNA wound in about 1.7 left-handed superhelical turns about the histone core with a total molecular weight of 206 kDa.

Results: Because of the experiment location in the laboratory module, it was not possible to access the DCAM units for an additional reload. Consequently, most of the nucleosome core particle crystals were produced on the launch pad; however, in a few experiments where the equilibration profiles were set for extended times, a large crystal of new morphology and space group was produced in microgravity. This is clearly a larger and more desirable crystal form for the structural determination. Analysis of this sample is awaiting synchrotron beam time.

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Neurophysin II/Vasopressin Complex

Purpose: To improve the diffraction quality of the crystals to aid in structural determination

The hormone vasopressin is synthesized and packaged in the posterior pituitary as a prohormone with its carrier neurophysin. Vasopressin, which has long been associated with cardiovascular function, has recently been shown (together with the related hormone oxytocin) to help orchestrate social and sexual relationships. Knowledge of this structure could provide information for basic understanding of human relationships, mental illness, and endocrinology.

Results: This experiment produced the largest crystal of neurophysin grown to date by any method, and crystals appear superior in optical perfection. An elaborate analysis has been performed, and details are forthcoming.

PROTEIN CRYSTAL GROWTH

Single-Locker Protein Crystal Growth (continued)

T7 RNA Polymerase

Purpose: To improve the diffraction quality of these crystals to aid in structural determination

T7 RNA polymerase is an enzyme responsible for reading the DNA code and translating it into RNA. The RNA then is used to make proteins that are necessary for cells to function. This structure will add to current knowledge of how life functions at its most minute level.

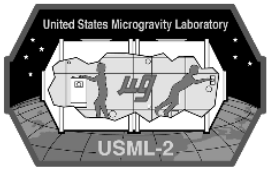
Results: The sample supply was exhausted during the extensive launch/scrub scenario.

Augmenter of Liver Regeneration (ALR) Protein

Purpose: To improve the diffraction quality of these crystals to aid in structural determination

ALR is a new liver growth factor that promotes liver regeneration after it is damaged or injured. The crystal structure of ALR will provide a road map for other researchers to use in better understanding the biochemical and physiological properties of this new class of growth factors and may open the door for new ways to treat liver diseases or to make liver transplants more successful.

Results: Large crystals with improved optical perfection were obtained; no additional information has been reported at this time.



PROTEIN CRYSTAL GROWTH

Commercial Protein Crystal Growth (CPCG)

PI: L.J. DeLucas

AFFILIATION: The University of Alabama at Birmingham, Alabama

PURPOSE: The general purpose of the Commercial Protein Crystal Growth experiments was to produce large, well-ordered crystals of various proteins under controlled conditions in microgravity.

METHOD: Commercial protein crystal growth experiments, sponsored by NASA's Code X, were conducted in Commercial Refrigerator/ Incubator Modules (CRIMs) in the middeck of the Shuttle. These experiments involved 1 CRIM that contained 60 vapor diffusion experiments at 4 ˚C and 1 CRIM that contained the Protein Crystallization Facility.

The Protein Crystallization Facility is a large-volume facility that used temperature change to induce the crystallization of a new form of recombinant human insulin. This project was flown in collaboration with Eli Lily. Because of the proprietary nature of this project, the results will be presented at a later date by Dr. Marianna Long.

RESULTS: Sixty vapor diffusion experiments were contained in Vapor Diffusion Apparatus (VDA) trays stored in a CRIM, set at 4 ˚C. The following list summarizes the results of these experiments.

PROTEIN RESULTS

Calcineurin Complex:
Many very small plates and cubes, less than 0.1 mm in length

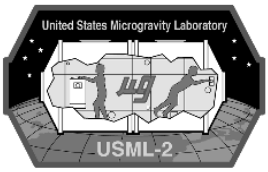
Epidermal Growth Factor Receptor:
Crystalline precipitate with some denatured protein

Trichomonas Vaginalis Ferredoxin:
Thin, rod-shaped crystals, up to 1 mm in length; most crystals were twinned and many drops contained heavy precipitate.

In general, few diffraction-sized crystals were obtained in the 4 ˚C VDA experiments. The disappointing results of these experiments are thought to be related to two factors: the significant launch delays and the unstable CRIM temperature during the mission. The multiple launch delays required that the loaded CRIM be installed in and removed from the orbiter several times and that some of the experiments be stored in the flight hardware for more that three weeks. During ascent, the CRIM temperature rose from the nominal temperature of 4 ˚C to 8.9 ˚C. The temperature then stabilized in the 4 to 6 ˚C range, but it spiked to 8.3 ˚C after landing.

PRELIMINARY CONCLUSIONS: Since the molecular stability of proteins decreases rapidly as a function of both time and temperature changes, it is difficult to differentiate between the effects of the launch delays and the temperature fluctuations on the 4 ˚C VDA experiments; however, clearly both factors had an impact on the success of these experiments.

SURFACE TENSION DRIVEN CONVECTION EXPERIMENT



PI: S. Ostrach

AFFILIATION: Case Western Reserve University, Cleveland, Ohio

PURPOSE: This experiment investigated the basic fluid mechanics and heat transfer of thermocapillary flows generated by temperature variations along free surfaces of liquids in low gravity. It determined when and how oscillatory thermocapillary flows were created.

METHOD: Thermocapillary flows were generated in cylindrical test chambers filled with low-viscosity silicone oil. Six modules were used to study two different heating modes and three different chamber diameters (1.2, 2, and 3 cm). A submerged heating system was used to study flows over a range of imposed temperature differences, while a carbon dioxide laser surface heating system was employed to study flows generated by various heat fluxes distributed across the surface of the liquid. A laser diode illuminated aluminum oxide particles suspended in the silicone oil, and the particle motion was recorded by a video camera. An infrared imaging system recorded oil surface temperature. An optical system (Ronchi system) was used to measure oil surface deformations and motions associated with oscillatory thermocapillary flows.

RESULTS: The STDCE completed all of its defined tests for the mission, several re-run tests to check data reproducibility, and additional tests with shallow test chambers. In all, 42 constant flux tests and 13 constant temperature tests were conducted and completed successfully, and oscillations were found in most of the tests. The hardware performed very well, and the quality of the video and digital data was excellent. It may take 2 years to analyze all the data. Preliminary analysis shows:

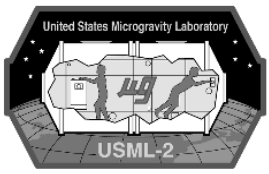
I. The critical temperature differences and heat fluxes for the onset of oscillations for the 1.2-cm chambers with flat free surfaces were close to those found in ground-based tests. For the 2- and 3-cm chambers, the critical values were much lower than those found on the ground, showing the effect of buoyancy in the latter. The space experiment provided a much larger parametric space than is possible on the ground without the effects of buoyancy.

II. The largest critical Marangoni number in the tests was nearly three times larger than that in ground-based tests using smaller chambers, which means there is no fixed critical Marangoni number to specify the onset of oscillations.

III. The critical values increased as the free surface was made more concave. In fact, the flow did not become oscillatory in the 1.2-cm chamber with a deep concave surface, even when the heater temperature was increased to the maximum allowed by the hardware.

IV. The critical heat fluxes increased when the chambers were shallow.

PRELIMINARY CONCLUSIONS: It is still too early to draw final conclusions. So far, the most important finding is that the Marangoni number is not the proper parameter to specify the onset of oscillations, which is consistent with the current theoretical model.



ZEOLITE CRYSTAL GROWTH

PI: **A. Sacco, Jr.**

AFFILIATION: Worcester Polytechnic Institute, Worcester, Massachusetts

PURPOSE: The USML-2 Zeolite Crystal Growth (ZCG) experiment attempted to establish a level of understanding of zeolite crystallization to allow control of both lattice defect concentration and crystallite size. Zeolite crystals are used in the chemical process industry as molecular filters, catalysts, and adsorbents. These three-dimensional crystalline materials can molecularly filter and, with proper selection of cations, adsorb and catalytically process molecules in a highly selective manner. Because of their small size (typically 2 to 8 μm), many qualities, such as structure, cation locations, and catalytic sites and locations of these sites are difficult to determine. Large zeolite crystals in the range of 500 to 1000 μm would greatly improve the understanding of the chemical and physical features of zeolites, resulting in the optimization of existing uses and the development of new uses. If large, well-characterized crystals with controlled defect concentrations could be made, they could be used as industrial membranes (a major improvement over existing industrial separation processes), as more effective scavengers for low-level nuclear waste materials, and as host materials (quantum confinement) for semiconductors. Theoretically, the microgravity environment of Earth orbit can provide a unique laboratory in which to learn how to control defect concentration and to grow large zeolite crystals in high yield.

Evaluation of the zeolite crystals grown on the First United States Microgravity Laboratory and Spacehab-1 indicate that most samples in which nucleation was controlled experienced enhanced growth and achieved a degree of crystal perfection as high or higher than any crystal produced on Earth. Several crystals on zeolite A appeared to be approaching the theoretically perfect silicon aluminum ratio, the first time such a ratio has ever been achieved. Area increases of 96% and volume increases of 175% over the Earth-grown control samples were observed for zeolite A. Area and volume increases for crystals of zeolite X increased 50% and 83% respectively, when compared to the best laboratory samples ever produced by the PI. On USML-2, the ZCG experiment continued to investigate techniques for crystallizing several different zeolites in low-Earth orbit.

METHOD: The ZCG experiment on USML-2 occupied 2 middeck lockers and consisted of 38 autoclaves that were activated and loaded into the ZCG furnace assembly. Zeolite synthesis begins with the initial mixing of the two source solutions (one aluminum-based and the other silicon-based), so the autoclaves were designed to be loaded on Earth for mixing in orbit. They also were designed for ease of installation into and removal from the furnace.

Each metal autoclave contained two chambers and a screw assembly. By turning the screw assembly with a powered screwdriver, the solution in one chamber was pressurized and forced into the main chamber. Turning the screw in the opposite direction pulled fluid back into the emptied chamber. By repeating this process several times, proper mixing of the two solutions was obtained. On USML-2, several different

ZEOLITE CRYSTAL GROWTH

nozzle designs and mixing aids were used. Experiments conducted in the Glovebox using clear autoclaves determined the proper number of times the fluids should be worked to ensure proper mixing for each design.

The furnace assembly consisted of 19 heater tubes/support structures, each holding two autoclaves, surrounded by insulation and an outer shell. The furnace automatically processed the multiple samples in three independently controlled temperature zones of 175 °C, 120 °C, and 100 °C.

RESULTS: The ZCG furnace performed nominally during the entire 12 days, 22 hours, and 25 minutes of operation. During the heatup of the outer furnace zone, furnace tubes 15, 16, and 17 lagged as much as 6 °C behind the rest of the furnace tubes in that zone. The reason for this and the effect, if any, it had on the flight samples are being investigated. Once the steady-state set-point was reached, all 19 tubes in all 3 zones held set-point temperatures to ± 1 °C. All 19 flight autoclaves and their associated ground-control autoclaves have been removed from the autoclaves, filtered, and stabilized for analysis.

Preliminary electron microscopy has been done on most of the flight and control samples. These preliminary findings indicate that the largest flight crystals of zeolite X are 25% to 50% larger in linear dimension than the ground controls and are, on average, 100% larger than those produced on USML-1 and Spacehab-1. Zeolite-ß was observed to grow to a similar size for both the ground-based and flight samples. No attempt was made to control the nucleation event for zeolite-ß; thus, the identical size of the two samples is not surprising. The silicate flown has shown a more well-defined morphology with significantly fewer intergrowths than the ground control. The size appears to be dependent somewhat on the form of the silica gel source and physical pretreatment. With one untreated silica source, the flight crystals are larger than the controls. In the other case (heat-treated), the flight crystals are of comparable size to the controls or are even slightly smaller. As with the USML-1 and Spacehab-1 results, if the nucleation event was not controlled, then the size of the space-grown crystals was not necessarily larger. Also, for those solutions where larger crystals formed, the particle size distribution was broader (based on optical analysis), and the average crystal size shifted to larger particle sizes, relative to the ground-based controls.

At present, the lattice defect concentrations have not been determined. This analysis will be performed over the next 6 to 9 months. In addition, no information on the samples flown for European Space Agency (ESA) has been given to Worcester Polytechnic Institute. A meeting will be scheduled later this spring to compare and discuss the results from both groups.

PRELIMINARY CONCLUSIONS: The preliminary conclusions indicate that the Zeolite Crystal Furnace worked nominally. In addition, for zeolites X and A, with few exceptions, the crystals from USML-2 are larger in size than their Earth-grown counterparts and are twice as large as those grown on previous flights. The results for zeolite-ß and silicate are consistent with no nucleation control. The results for ESA samples are not known at this time. Analysis will continue to determine the effect on lattice defect concentration of space processing.

Orbital Acceleration Research Experiment (OARE)

PROJECT MANAGER: W. Wagar

AFFILIATION: NASA, Lewis Research Center, Cleveland, Ohio

PURPOSE: The Orbital Acceleration Research Experiment monitors and records onboard accelerations and vibrations experienced during orbital flight so that researchers can use the data to assess the influence of Shuttle accelerations on their experiments. The OARE instrument is part of the Principal Investigator Microgravity Services (PIMS) project that supports PIs in the evaluation of the effects of varying acceleration levels on their experiments. A summary report will be prepared by PIMS personnnel that will include the data collected by the OARE instrument and will furnish interested experiment investigators with a guide to evaluating the acceleration environment during the USML-2 mission.

METHOD: The OARE instrument measures low-level accelerations in the frequency range below 1 Hertz down to essentially steady-state. It was mounted near the center of gravity of the orbiter vehicle outside the Spacelab module and in the payload bay. OARE was activated at launch minus 5 hours. Quasistatic data were recorded during ascent, throughout the mission, and upon re-entry. For the first time, quasistatic data also were downlinked and were available to the PIs in the Payload Operations Control Center through video, as well as on three sites on the World Wide Web (the USML-2, OARE, and PIMS sites).

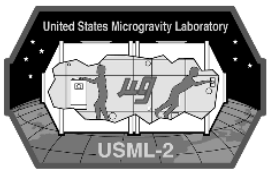
RESULTS: Measured OARE quasistatic data were compared to predicted data obtained through the Microgravity Acceleration Workstation model. This comparison was done in real time and showed very good agreement between the two. The PIMS group also supported the PIs during the mission by providing data on transient effects, such as payload bay door opening/closing, water dumps, and thruster firings. During the CGF attitude, the PIMS group worked closely with the PIs to provide quasistatic data at the CGF sample melt site. Mission management also relied on the OARE data when faced with performing unscheduled maneuvers and the unexpected payload bay door opening/closing that occurred because of a tire cooling problem.

During the mission, the bias and scale factor calculations for the OARE data were approximate. This is because, for accurate bias and data calculation, investigators require data from the entire length of the mission to curve fit. The raw data obtained from the OARE then were compensated for bias and scale. One additional difficulty during the mission was that, because of Loss of Signal (LOS) time, almost half of the data was not downlinked, although the data still were being recorded on board; hence, OARE data during the LOS periods could not be provided to the PIs, and the real-time bias and scale calculations were approximate.

Orbital Acceleration Research Experiment (continued)

Bias and scale factor calculations have been performed post-mission using the OARE data from the entire mission. OARE data have been compensated for these. The data are now provided to the PIs by means of file transfer process or by access to the OARE Web page. The data provided are those measured at the OARE site in orbiter body coordinates versus time.

PRELIMINARY CONCLUSIONS: Data from the OARE have been processed and made available to the science community. The real-time preliminary OARE data provided to the PIs during the mission now can be replaced by the final OARE data. The OARE data on the World Wide Web and the file server are those measured at the OARE location; however, they can be transformed easily to other experiment sites. A mission summary report is being prepared to describe the microgravity environment of the mission. In this report, details of the effects of various phenomena, as recorded by OARE and the other accelerometers, will be discussed.



MEASURING MICROGRAVITY

Space Acceleration Measurement System (SAMS)

PROJECT MANAGER: R. Sicker

AFFILIATION: NASA, Lewis Research Center, Cleveland, Ohio

PURPOSE: The Space Acceleration Measurement System monitors and records onboard accelerations and vibrations experienced during orbital flight so that researchers can use the data to assess the influence of Shuttle accelerations on their experiments. The SAMS instrument is part of the Principal Investigator Microgravity Services (PIMS) project that supports PIs in the evaluation of the effects of varying accelerations levels on their experiments. A summary report will be prepared by PIMS personnel that will include the data collected by the SAMS instrument and will furnish interested experiment investigators with a guide to evaluating the acceleration environment during the USML-2 mission.

METHOD: SAMS measured the low-gravity environment of the orbiter *Columbia* during the USML-2 mission. The three SAMS units have the capability of measuring low-level accelerations from about 0.01 Hertz up to a maximum cut-off frequency of 100 Hertz. SAMS sensors were mounted at the Surface Tension Driven Convection Experiment (Rack 3; 5 Hertz), Glovebox (Rack 12; 25 Hertz), and Crystal Growth Furnace (Rack 9; 2.5 Hertz) experiment sites. SAMS was activated at 00/06:48 Mission Elapsed Time and recorded data continuously throughout the mission. The SAMS data were not downlinked during the mission but were recorded on board on optical disks.

RESULTS: The SAMS data have been processed for all three sensor heads. Investigators are working to provide this information on the World Wide Web and on the beech server for file transfer. This will be done in the near future. PIs then will have access to the x, y, and z data from each head versus time.

PRELIMINARY CONCLUSIONS: A mission summary report is being prepared to describe the microgravity environment of the USML-2 mission. In this report, details of the effects of various phenomena, such as those recorded by the SAMS instrument, will be discussed.

MEASURING MICROGRAVITY

Three-Dimensional Microgravity Accelerometer (3DMA)

PI: J. Bijvoet

AFFILIATION: University of Alabama in Huntsville, Alabama

PURPOSE: The objectives of the Three-Dimensional Microgravity Accelerometer project were twofold:

I. Provide and validate a full-performance, low-cost, marketable 3DMA system in support of the objectives of the Centers for Commercial Development of Space

II. Validate the following advanced approaches:

A. Downlink of real-time acceleration data from several onboard locations to the ground in support of the Principal Investigators and the Mission Scientist

B. Simultaneous and continuous provision of three frequency ranges from each accelerometer

C. Fully automatic data recording on board not requiring routine crew actions, except for occasional performance checks

D. Adequate measurement of the quasi-stationary, very-low-frequency, low-level accelerations with a very-low-cost system using Invertible Accelerometers

E. Novel "panoramic" multi-location data display method and provision of acceleration frequency spectrum on demand of any selected channel.

METHOD: Three-dimensional sensors were located at four locations: in racks 5 and 8, on the subfloor, and in the Invertible Accelerometers housed in the Central Unit. The Central Unit was located in the center aisle and also housed data processing and onboard data recording systems. Real-time data were sent to the ground via the Spacelab High Rate Multiplexer. During periods of Loss of Signal, all data were recorded by the Spacelab High Data Rate Recorder and were relayed later to the ground. All data were displayed and recorded on the ground at the Huntsville Operations Support Center (HOSC). Microgravity data from any selected sensor were broadcast in the HOSC to PIs on the video matrix.

The 3DMA data processing system on the ground provided panoramic real-time display of all data and real-time absolute-g calculations from the Invertible Accelerometers. Recorded data will be made available to PIs upon request on a 4-mm DAT tape. The multiple sensor/multiple channel 5-minute displays allow quick scanning of the mission. Upon request, frequency and spectra data from selected locations can be provided to the PIs.

Three-Dimensional Microgravity Accelerometer (continued)

RESULTS: The significance of having real-time acceleration data available to the science teams on the ground was conclusively demonstrated. The starts of some experiment sample runs, such as those for the Surface Tension Driven Convection Experiment, were timed to the quieting of acceleration disturbances as measured by the 3DMA and displayed to the scientists on the ground. In addition, steps were taken to provide the 3DMA acceleration data back to the crew aboard the Shuttle.

The Mission Scientist was alerted to harmful vibrations of about 4×10^{-4}g caused by the Glovebox fan. The Glovebox was located in a rack across the center aisle from the 3DMA Remote Units. The fan axis orientation was determined from the data and correlated with the fan design. As a result, the use of the fan was reduced for the remainder of the mission in the interest of the success of other experiments.

MEASURING MICROGRAVITY

Suppression of Transient Events by Levitation (STABLE)

PI: G.S. Nurre and D.L. Edberg

AFFILIATION: NASA Marshall Space Flight Center and McDonnell Douglas Aerospace, Huntsville, Alabama

PURPOSE: Microgravity science payloads can be extremely sensitive to vibrations from machinery, acoustics, ventilation, and crew activity. STABLE is a vibration isolation system designed to protect payloads from these disturbances for periods of 30 days or more. Initial flight data analyses show that the acceleration environment aboard STABLE's isolated platform was attenuated by a factor of more than 25 across the band from 0.01 to 300 Hertz.

METHOD: Built under a cooperative agreement by engineers and scientists from McDonnell Douglas Aerospace (MDA), Huntington Beach, California, and Marshall Space Flight Center (MSFC), Alabama, the entire isolation system fit in a single middeck locker and provided an uninterrupted microgravity environment for its own payload, a fluids experiment dubbed CHUCK.

STABLE comprises six highly sensitive accelerometers mounted on a free-floating platform contained within the locker box. The MDA-designed control system feeds the acceleration signals to a set of non-contacting actuators to cancel any measured acceleration, while the MSFC-designed positioning system keeps the platform centered between disturbances. Power, control, data, and video signals were transmitted between the base and the isolated platform using soft umbilicals. For launch and landing, the floating platform was locked down by an MSFC-designed caging mechanism, which was manually activated by the crew.

Three accelerometers mounted at the rear of the locker measured the base acceleration environment, while the six accelerometers aboard the isolated platform measured the isolated acceleration environment. A Payload and General Support Computer (PGSC) recorded the data to disk during the mission. More than 4 gigabytes of data were recorded during the mission.

RESULTS: Figure 1 is an acceleration time history during normal crew activity at Mission Elapsed Time 12:18:10:00. Base accelerations of up to 1500 mg were measured, but accelerations on the isolated platform were near the accelerometer noise floor, peaking at 50 mg. Figure 2 shows these same acceleration time histories on an expanded scale. The root-mean-square (RMS) environment was reduced from 546 mg at the base to 21 mg on the isolated platform. Figure 3 shows the base and isolated platform RMS acceleration in 1/3 octave bands and compares them with the requirement for the International Space Station.

Suppression of Transient Events by Levitation (continued)

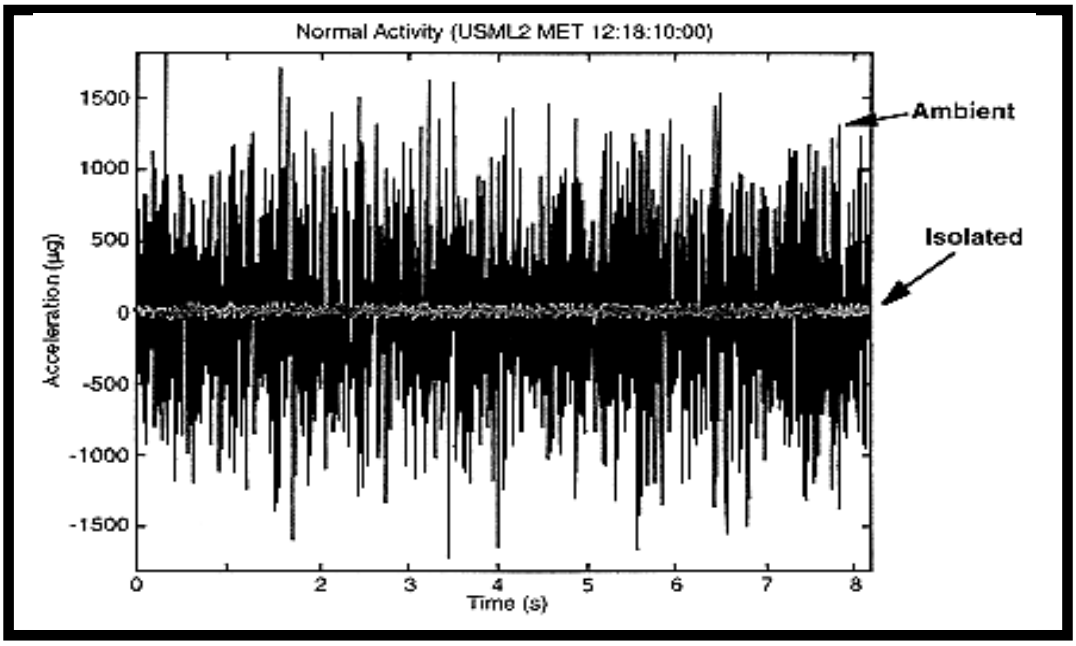


Figure 1. This time history shows that STABLE reduced the acceleration environment on the isolated platform significantly below ambient.

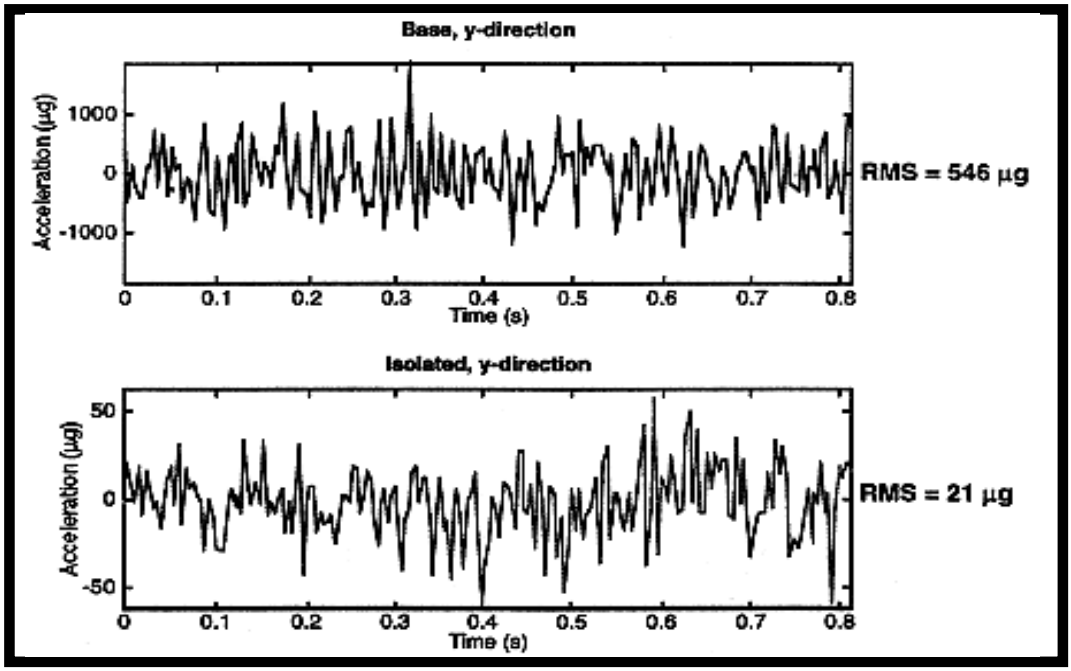


Figure 2. Expanded scale of the time history of normal crew activity shows peak accelerations on the isolated platform less than 50 mg, compared to more than 1000 mg on the base.

Suppression of Transient Events by Levitation (continued)

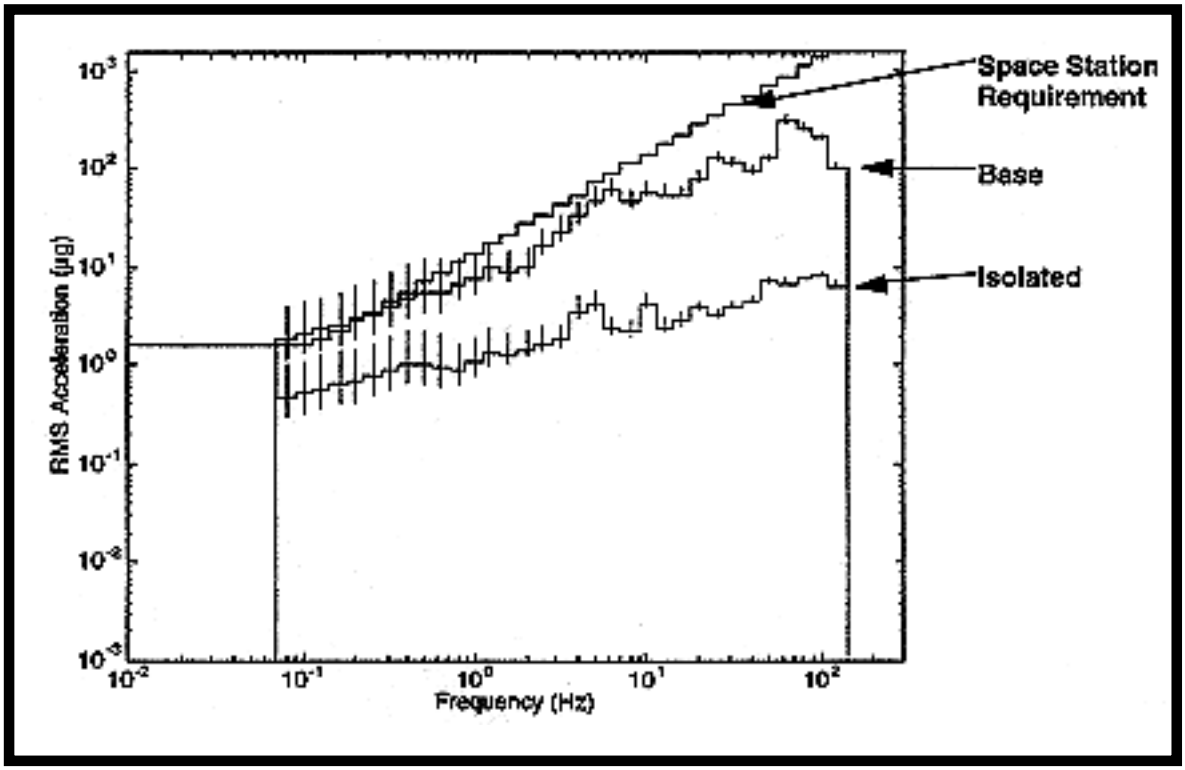


Figure 3. Normal crew activity 1/3 octave band RMS spectral analysis shows accelerations on the isolated platform were substantially below that of the base (vertical lines are error bars).

PRELIMINARY CONCLUSIONS: Data reduction efforts will continue during 1996; however, initial results show that STABLE provided a significantly reduced acceleration environment aboard Spacelab. The success of the STABLE demonstration opens new areas for research in a true, uninterrupted microgravity environment.

Based on this success, plans are underway to enhance the STABLE hardware with a controlled-acceleration mode, whereby the science payload can be subjected to a user-selected acceleration environment, so that the STABLE platform may serve as a microgravity shaker, as well as a true microgravity platform. This promises to open new possibilities for scientific investigation in the area of acceleration sensitivity thresholds in materials and fluids science.

Microgravity Acceleration Workstation (MAWS)

LEAD ENGINEER: L. French

AFFILIATION: Teledyne Brown Engineering, Huntsville, Alabama

PURPOSE: The Microgravity Acceleration Workstation operated during the USML-2 mission to predict the microgravity environment of the Shuttle. These models were used to help scientists and mission planners schedule and replan experiment objectives.

METHOD: The MAWS project accessed and processed telemetry data from the orbiter to predict the nominal microgravity environment. These models were then compared to the actual acceleration data being gathered by the OARE instrument, and updates were made.

RESULTS: The MAWS software and hardware demonstrated the maturity of the system by operating continuously during the USML-2 mission. No significant system anomalies were experienced during the mission operations. MAWS operations during USML-2 confirmed the system to be a robust telemetry processing system, which may be applied to a broad range of Spacelab microgravity data acquisition/ processing problems. The quality of the analytical data is demonstrated by the agreement with the OARE accelerometer, which was typically within 50 nano-gs for a period of several hours following an OARE calibration event. Like any sensor, the OARE device tended to drift with time, which was easily identified by the MAWS system.

MAWS successfully acquired and processed real-time microgravity acceleration data during the entire mission. Attitude corrections were made real time based on MAWS predictions of the acceleration environment. These attitude changes were made in conjunction with the off-line 6-degree-of-freedom model SAMSON. Verified initial conditions from the MAWS system were confirmed using OARE. These conditions were then used as inputs to SAMSON to determine the optimal attitude for CGF. The predicted SAMSON microgravity conditions were duplicated during the actual CGF operations period, exactly as predicted by SAMSON.

MAWS acquired huge amounts of USML-2 microgravity and dynamics data. These data may be useful to the USML-2 scientists to provide correlation of the environment with the science samples to see if the environment in any way affected the results. This large database of information can be used to assist in diagnosing any acceleration mysteries and to improve science data acquisition for future microgravity missions. Of particular note was the historic "first" of recomputing the optimal attitude for a microgravity experiment based on real-time information and successfully observing the conditions exactly as predicted.

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ASTROCULTURE™

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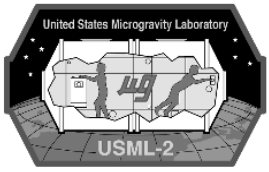

CO-INVESTIGATORS

Crystal Growth Furnace

Orbital Processing of High-Quality Cadmium Zinc Telluride (CdZnTe) Compound Semiconductors

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J.I. Alexander	University of Alabama in Huntsville
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J. Moosbrugger	Clarkson University
M. Dudley	SUNY-Stony Brook

The Study of Dopant Segregation Behavior During the Crystal Growth of GaAs (Gallium Arsenide) in Microgravity

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D. Watring	NASA/MSFC
J. Kafalas	Viable Systems
M. Kaforey	Case Western Reserve University

Drop Physics Module

Drop Dynamics Experiment

C.P. Lee	Vanderbilt University
A. Anilkumar	Vanderbilt University
E. Trinh	NASA/JPL

Science and Technology of Surface-Controlled Phenomena

G. Holt	NASA/JPL

Geophysical Fluid Flow Cell Experiment

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F. Leslie	NASA/MSFC
T. Miller	NASA/MSFC
D. Hathaway	NASA/MSFC
G. Glatzmaier	Los Alamos National Laboratory
D. Ohlsen	University of Colorado

Glovebox Investigations

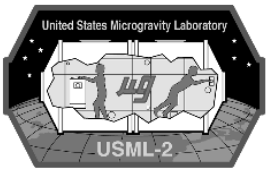
Colloidal Disorder-Order Transitions

W. Russel	Princeton University

Interface Configuration Experiment

R. Finn	Stanford University
M. Weislogel	NASA/LeRC

CO-INVESTIGATORS



Protein Crystal Growth-Glovebox
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Oscillatory Thermocapillary Flow Experiment

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Fiber Supported Droplet Combustion

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J. Haggard	NASA/LeRC
V. Nayagam	NASA/LeRC
B. Shaw	University of California, Davis

Protein Crystal Growth

Commercial Protein Crystal Growth

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Single Locker Protein Crystal Growth

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J. Rose	University of Georgia
J. Rosenberg	University of Pittsburgh
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J. Harp	Oak Ridge National Laboratories

Surface Tension Driven Convection Experiment

Y. Kamotani	Case Western Reserve University

Zeolite Crystal Growth

A. Dixon	Worcester Polytechnic Institute
R. Thompson	Worcester Polytechnic Institute

HARDWARE DEVELOPERS

Advanced Protein Crystallization Facility
K. Fuhrman ESA/ESTEC

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D. Schaefer NASA/MSFC

Drop Physics Module
D. Gallagher NASA/JPL

Geophysical Fluid Flow Cell
G. Hall NASA/MSFC

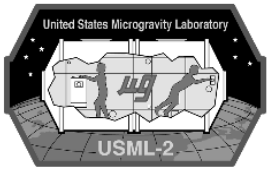
Glovebox
C. Darty NASA/MSFC

Single-Locker Protein Crystal Growth Apparatus
B. Herren NASA/MSFC

Commercial Protein Crystal Growth Apparatus
J. Nordness Center for Macromolecular Crystallography

Surface Tension Driven Convection Experiment Apparatus
T. Jacobson NASA/LeRC

Zeolite Crystal Growth Facility
L. McCauley Battelle Advanced Materials Center for the Commercial Development of Space



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